

THE MUSCLE CONTENT OF THE LOWER OESOPHAGUS OF MAN¹

L. B. AREY AND M. J. TREMAINE

Northwestern University Medical School

The composition and arrangement of the muscular coat of the oesophagus vary widely in mammals. Most possess two layers, but some have three (carnivores; rabbit); others ordinarily three, but sometimes a part of a fourth (equidae; ruminants); while still others have four layers regularly (swine). Furthermore, the purely smooth muscle content of the oesophagus which is characteristic of amphibia, reptiles, birds, and monotremes is encroached upon in all higher mammals by skeletal muscle fibers descending from the pharynx to different levels. These various conditions are summarized by Oppel (1897) and others. In a few mammals (some marsupials and cetaceans) skeletal fibers are limited to the upper part of the oesophagus, while in the horse, cat, and bat they pass below the middle level. Most other mammals have skeletal fibers distributed throughout the entire oesophageal length, and there are even instances of a continuation onto the stomach wall (dormouse).

In man it is usually considered (Schumacher, '27, et al.) that the upper fourth of the oesophagus contains skeletal muscle in both its layers, while in the second quarter the smooth muscle becomes plentiful and progressively replaces the skeletal fibers until the lower half or third consists of smooth muscle only. Schwann (1836) long ago made pioneer observations on the limitation of striate fibers to the superior half of the oesophagus. Somewhat later, Welcker and Schweigger-Seidel (1861) presented a quantitative report on

¹ Contribution no. 192.

the proportions of smooth and striate muscle at twelve different levels of four oesophagi. They found no skeletal muscle in the lower two quarters, and with this conclusion other observers have agreed from time to time, including Goetsch ('09), who studied carefully two specimens. Nevertheless, some exceptions to these broad conclusions have been cited. Individual bundles of smooth muscle were early described by Klein (1868) at the beginning of the human oesophagus, and Schaffer (1897) later found them even in the region of the pharynx. In addition, certain workers have claimed the occurrence of some striate muscle descending into the lower oesophageal third and extending even to the cardiac orifice. These claims must be reviewed briefly, since it is with such statements, discrepant with the more generally accepted belief, that the present communication deals.

The earliest record that skeletal muscle fibers extend even to the cardia were perhaps furnished by Ficinus (1836) and by Valentin (1837), although it is said that the latter subsequently identified smooth muscle in the lower oesophagus.

Gillette (1872) examined twenty oesophagi microscopically and found skeletal fibers exclusively in the superior part and smooth fibers exclusively in the middle portion. However, in the inferior part both types of muscle fibers were said to be encountered, although the striate component was considerably less abundant than in the upper level. Since these striate fibers were recorded as occupying a superficial position in the oesophageal wall, both at and below the diaphragmatic orifice, Gillette thought that their origin could be traced from the pillars of the diaphragm, whence they constituted a third muscular coat to the oesophagus. An extent to a level as low as the cardia he held as extremely rare.

Twenty years later, Coakley (1892), seemingly unaware of predecessors, reported somewhat similarly. His observations may be assumed to deal with man, although no direct statements as to materials are furnished. Like Gillette, this observer maintained that striate fibers, tracing origin from the pillars of the diaphragm, intermingle with the smooth muscle

fibers of the abdominal portion of the oesophagus. But those nearest the diaphragm are reported as occupying a circular disposition with a maximum concentration in the inner, or circular, muscle layer. These striate fibers of the circular layer, at first nearly equaling the smooth fibers in 'number' (*sic*), rapidly diminish at lower levels; here they become arranged as scattered bundles and all disappear close to the cardiac orifice. On the other hand, the less abundant striate fibers in the outer, or longitudinal, layer change from a circular distribution to an oblique course and finally to a longitudinal direction; descending without great diminution in numbers, some even continue for a short distance onto the cardiac stomach. Furthermore, a transition in structure is described, those striate fibers at the level of the diaphragm having a typical appearance, while nearer the stomach they become smaller in size and more feebly banded; thus they are looked upon as intermediate between striate and smooth elements.

Recently, Hartridge and Haynes ('30) have reiterated the same principal thesis, stating that: "In the lower part of the viscus the striated fibers are gradually replaced by smooth muscle so that as the stomach is approached more and more of the musculature is smooth. Even at the cardiac orifice, however, a few striated fibers may be found, the proportion of smooth to striated fibers at any given level varying with the individual." An accompanying drawing through the 'lower oesophagus' shows fascicles composed of each muscle type; in the region depicted the skeletal fibers occupy a slightly greater total area.

OBSERVATIONS

In order to obtain additional information on this seemingly simple morphological point, the present writers have collected oesophagi from routine dissecting-room cadavers which had been embalmed with the common formol-alcohol-carbolic solution. The lower one-third of each oesophagus was removed and divided transversely into four equal portions, which were

then subjected to the paraffin technique. Sections were cut from the caudal surface of each block, the lowest level being close to the cardiac orifice. Examination of representative hematoxylin and eosin sections proved that some specimens were unsuitable for study, but seventy-four oesophagi were selected as adequate.² The preservation of these was reasonably good, and in some instances astonishingly so; shrinkage was the chief technical defect in the muscular coat.

Search was then made for striate muscle, either in the form of individual fibers or fascicles, that might be located at any of the four levels of the lower oesophageal third. Only one of the seventy-four oesophagi contained skeletal muscle in this segment.³ In this specimen the skeletal fibers were so abundant, even close to the cardiac orifice, that it may have been that such elements extended onto the stomach wall, although no information on this point was obtained. These fibers, at least in the longitudinal coat where transverse sections would show the condition plainest, were for the most part grouped into fascicles of various sizes. Only rarely could single striate fibers be seen isolated within smooth muscle fascicles, whereas the reverse condition was never found. There were some long stretches in the longitudinal coat occupied almost exclusively by skeletal muscle, and similar extents composed preponderatingly of smooth muscle. In still other regions fascicles of both types intermingled freely. In the circular coat the majority of striate fibers were located nearer the periphery where the intermingling was general. Only in a limited sector of this coat as a whole did skeletal muscle fail. A quantitative determination of the relative amounts of skeletal and smooth fibers at the lowest level was made by tracing the outlines of all the muscle fascicles on cardboard and then weighing the two groups.⁴ In this way the following ratios of distribution were established:

² In eleven of the seventy-four specimens the segment of the oesophagus below the diaphragm was the only portion obtained.

³ The subject from which this oesophagus came was a male negro, 21 years old, who was 6 feet 2 inches tall and weighed 150 pounds.

⁴ There was little difference to the eye between the muscle content at the lowest or highest level of the lower segment of this oesophagus.

External, or longitudinal, layer:

Skeletal muscle,	63.4 per cent
Smooth muscle,	36.6 per cent

Internal, or circular, layer:

Skeletal muscle,	20.1 per cent
Smooth muscle,	79.9 per cent

It is not at all astonishing that an occasional variation should occur in the downward extent of skeletal muscle on the wall of the oesophagus, especially when other Primates are characterized by a full-length distribution. Indeed, the customary uniformity of distribution is really a much more remarkable phenomenon. From our results it may be inferred that the presence of striate muscle in the lower oesophagus is not typical and must be considered wholly anomalous. The contrary conclusion of other workers already cited is not easy to explain. Early errors, as by Ficinus (1836) and by Valentin (1837), are perhaps to be expected, but the reports of Gillette (1872) and Coakley (1892) upon a diaphragmatic contribution to the oesophagus are indeed puzzling. Both accounts contain the same general idea, although differing in details, and neither view receives any support from our preparations. What these observers saw and attempted to describe as a regular feature in the composition of the lower oesophagus is difficult to imagine. That there are racial differences is conceivable, but not very probable. Our material doubtless provides a fair representation of the racial composition of the cosmopolitan city of Chicago. Although the one specimen showing striate muscle in the lower oesophagus was a negro, yet a considerable number of other negroes who lacked this feature must have been included in the series. One hesitates to suggest the failure of others to distinguish properly between the two types of muscle; nevertheless, it is true that shrinkage can make fascicles of smooth muscle resemble superficially the skeletal type, as some of our material shows.

CONCLUSIONS

The lower thirds of seventy-four human oesophagi were examined microscopically at four different levels for skeletal muscle fibers.

Only one individual (a negro) contained any skeletal muscle at these lower oesophageal levels. This striate muscle content occurred as fascicles which amounted to 63 per cent of the longitudinal muscle coat and 20 per cent of the circular coat. It is possible that a continuation of skeletal fibers onto the stomach wall occurred.

Contrary to certain statements which have been made from time to time, the occurrence of skeletal muscle in the lower oesophagus of man must be considered anomalous.

LITERATURE CITED

- COAKLEY, C. G. 1892 The arrangement of the muscular fibers of the oesophagus. Res. of Loomis Lab. (Univ. of City of New York), vol. 2, pp. 113-114.
- FIGINUS, H. R. 1836 De fibrae muscularis forma et structura. Leipzig.
- GILLETTE, E. P. 1872 Description et structure de la tunique musculaire de l'oesophage chez l'homme et chez les animaux. J. de l'anat. et de la physiol., T. 8, pp. 617-644.
- GOETSCH, E. 1910 The structure of the mammalian oesophagus. Am. J. Anat., vol. 10, pp. 1-40.
- HARTRIDGE, H., AND F. HAYNES 1930 Histology for medical students. Oxford Univ. Press, London.
- KLEIN, E. 1868 Über die Vertheilung der Muskeln des Oesophagus. Sitzungs. d. k. Akad. d. Wissensch. zu Wien (math.-naturw. Kl.), Bd. 57, Abt. 1, S. 1111-1121.
- OPPEL, A. 1897 Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere. Teil 2, Schlund und Darm. Fischer, Jena.
- SCHAFFER, J. 1897 Beiträge zur Histologie menschlicher Organe. Sitzungs. d. k. Akad. d. Wissensch. zu Wien (math.-naturw. Kl.), Bd. 106, Abt. 3, S. 353-455.
- SCHUMACHER, S. 1927 Die Speiseröhre. In Mollendorff's Handbuch der mikroskopischen Anatomie des Menschen. Bd. 5, Teil 1, S. 301-336. Springer, Berlin.
- SCHWANN, T. 1836 Cited in Jahresbericht über die Fortschritte der anatomisch-physiologischen Wissenschaften im Jahre 1835. Arch. f. Anat., Physiol. u. wissensch. Med., Jahrgang 1836.
- VALENTIN, G. 1837 Repertorium für Anatomie und Physiologie, Bd. 2, S. 86.
- WELCKER, H., AND F. SCHWEIGGER-SEIDEL 1861 Verbreitungsgrenzen der quergestreiften und glatten Muskulatur im menschlichen Schlunde. Arch. f. path. Anat. u. Physiol., Bd. 21, S. 455-456.

THE PRODUCTION OF MUCIFIED CELLS IN THE VAGINAL EPITHELIUM OF CERTAIN RODENTS BY OESTRIN AND BY CORPUS LUTEUM EXTRACTS

ROLAND K. MEYER¹ AND WILLARD M. ALLEN²

*Department of Anatomy and Department of Pathology, School of Medicine and
Dentistry, University of Rochester*

THREE PLATES (FOURTEEN FIGURES)

INTRODUCTION

It is a generally observed fact that during pregnancy and pseudopregnancy in many of the rodents the vaginal epithelium is changed from the squamous to the mucous type, but the factors responsible for this change are as yet largely obscure.³ The experimental production of this picture was first accomplished in 1924 by Courrier. He found that the injection of follicular fluid into both immature and castrated adult guinea-pigs resulted in mucification of the vaginal epithelium. A similar result was subsequently obtained by the administration of corpus luteum extracts in castrated rats by Hisaw, Meyer, and Weichert ('28) and in castrated mice by Weisner and Patel ('29). Both of these latter groups of workers considered the mucification produced by their extracts due to a specific hormone of the corpus luteum. Numerous others (Clausberg, '30; Fels, '31; Harris and Newman, '31; Meyer and Allen, '32) have also shown that corpus luteum extracts, regardless of whether they are made by the acid

¹ National Research Council Fellow in the Biological Sciences.

² National Research Council Fellow in the Medical Sciences.

³ Retterer (1892) and Lataste (1892) reported the presence of mucified cells in the vagina of the dog, rabbit, guinea-pig, and mouse, and Long and Evans ('22) have shown that this kind of epithelium is found in the vagina of the rat during pseudopregnancy and pregnancy.

alcohol method (Fevold, Hisaw, and Meyer, '30) or by the neutral alcohol method (Corner and Allen, '29) will produce mucification in mice if a proper dose is used. It is not reasonable, however, to assume without question that the mucification produced by these extracts is due to a specific hormone of the corpus luteum, since the extracts used by these workers contained oestrin. On the contrary, it is more than likely that their results were due in part at least to the presence of oestrin; especially is this probable since the experiments of Courrier have shown that mucification can be produced by follicular fluid alone, and also since Selle ('22) has already demonstrated that mucification appears before the first oestrus in the pubertal guinea-pig and shortly before every oestrus thereafter. Thus, even in the normal guinea-pig mucification of the vaginal epithelium is found at a time when the corpus luteum is either non-existent or old and probably non-functional. As further evidence in favor of this supposition, Robson ('31) and Robson and Wiesner ('31) have demonstrated that mucification can be produced by subcornifying doses of oestrin and that progesterin is not necessary for this reaction, since corpus luteum extracts in which the progesterin had been inactivated by treatment with alkali still produce mucification. Their experiments did not prove, of course, that oestrin is necessarily the substance responsible for mucification, since their oestrin-containing extracts were crude, but they suggest that the final step necessary to demonstrate such a relation is the injection of chemically pure oestrin.

We (Meyer and Allen, '32) have published certain preliminary data on the effect of oestrin, Theelin and corpus luteum extracts on the vagina. In this paper we shall present these data in detail, and especially would we draw attention to the results obtained with Theelin, since they add a most convincing sequel to the experiments of Robson and Wiesner mentioned above.

PRODUCTION OF MUCIFIED CELLS IN THE VAGINAL EPITHELIUM
OF CASTRATED MICE BY CORPUS LUTEUM EXTRACTS

Some of the extracts used in this study were prepared from the corpora lutea of the sow according to the method of Allen ('32) and others according to that of Fevold, Hisaw, and Meyer ('30). The latter method was not followed exactly as we have found that both progestin and oestrin can be extracted with ethyl ether from the sludge which results from the evaporation of the neutralized acid alcohol extract of the corpora lutea. Fevold, Hisaw, and Leonard, as stated in a more recent paper ('32), have also found that progestin is ether soluble, thus agreeing with the results obtained by other workers. The progestin content of the corpus luteum extracts was assayed on castrated adult female rabbits according to the method described by Corner and Allen ('29), the minimum amount of the extract required to produce a +++ reaction in the uterus of the test rabbit being considered as one rabbit unit (Rb.u.)⁴ of progestin. The mice were castrated, brought into heat with Theelin, and then injected for the next 8 days with the corpus luteum extract. Vaginal smears were taken daily and the animals were sacrificed on the ninth day.

The results obtained (table 1) demonstrate that mucification of the vaginal epithelium may be produced with varying amounts of corpus luteum extracts and that when the amount of corpus luteum extract is sufficiently increased beyond that amount required for a +++ mucification, stratification and even cornification of the vaginal epithelium results (mice A8, A1, A13, table 1).⁵ It will also be noted that a +++ mucification can be produced with a large range of dosage. For example, preparation no. 88 produced a +++ with the equivalent of 45 gm. of fresh tissue and exactly the same degree of mucification with 2 times and 4 times that dose, and preparation no. 89 produced a +++ with the equivalent of

⁴ This abbreviation for 'rabbit unit' was first used by Leonard, Hisaw, and Fevold ('32), and we suggest its general adoption.

⁵ Figures 11, 12, and 13 illustrate the degrees of mucification designated by a +, ++, and +++, respectively.

21 gm. of fresh tissue and the same result with 3 times that dose. In a subsequent part of this paper it is shown that a +++ mucification is not produced over a wide range of dosage when Theelin is used. The significance of these results will be discussed in a forthcoming paper. Suffice it

TABLE 1
*Adult castrated female mice injected with corpus luteum extracts
containing progestin*

PREP. NO.	MOUSE NO.	FRESH TISSUE EQUIVALENT	SOLIDS	PROGESTIN	HISTOLOGICAL FINDINGS IN VAGINA AT AUTOPSY
		<i>Grams</i>	<i>Grams</i>	<i>Eb. U.</i>	
87	B 25	4.6	0.077	0.16	0 mucification
87	A 10	18.6	0.308	0.66	+++ mucification
87	B 52	37.2	0.616	1.32	+++ mucification
87	B 51	74.4	1.232	2.64	Stratification
A-7	B 1	5.9		0.21	+ mucification
A-7	A 17	11.8		0.42	+ mucification
A-7	A 18	23.6		0.83	+++ mucification
A-7	A 8	23.6		0.83	+++ mucification
A-7	A 1	28.3		1.00	Stratification. Slight mucification above
A-7	A 13 ¹	23.6		0.83	Typical heat smears on fifth and sixth days
88	A 7	11.2	0.011	0.3	0 mucification
88	B 29	22.5	0.022	0.7	++ mucification
88	B 28	45	0.044	1.3	+++ mucification
88	B 49	90	0.088	2.6	+++ mucification
88	B 53	180	0.176	5.3	+++ mucification
89	B 32	5.3	0.006	0.15	0 mucification
89	B 27	10.6	0.013	0.3	>+++<+++ mucification
89	B 26	21.2	0.027	0.6	+++ mucification
89	B 31	63.6	0.081	1.8	+++ mucification
89	B 54	84.8	0.108	2.4	Stratification. Slight mucification above

¹ Injected for 4 days only. All other animals injected for 8 days.

for the present to state that we believe these results indicate that corpus luteum extracts can inhibit the power of oestrin to cornify the vaginal epithelium of the mouse.

Thus, mucification of the vaginal epithelium of the castrated mouse may be produced with extracts of the corpus luteum containing both progestin and oestrin. An increase in the

amount of mucification can be produced by increasing the amount of corpus luteum extract. Stratification and cornification of the vaginal epithelium results if the amount of corpus luteum extract administered is sufficiently increased beyond the maximum amount required to produce +++ mucification.

PRODUCTION OF MUCIFIED CELLS IN THE VAGINAL EPITHELIUM
OF CASTRATED MICE BY THEELIN

It has been demonstrated in the first part of this paper that mucification of the vaginal epithelium of the castrated mouse may be produced by extracts of corpora lutea of the sow which contain both progesterin and oestrin. The question arises whether the results obtained were produced by the oestrin present in the extracts, by progesterin, by a combination of oestrin and progesterin, or by a third hormone. It has been shown by the researches of Robson and Wiesner ('31) that mucification can be produced in the vaginal epithelium of castrated mice by extracts of corpora lutea which contain no progesterin (the progesterin having been destroyed by alkali treatment) and also by crude extracts of follicular fluid. However, the extracts used by Robson and Wiesner were crude and it cannot be concluded with certainty whether their results were obtained by oestrin or some other substance. Therefore we decided to carry out similar experiments using pure oestrogenic hormone (Theelin).⁶ Accordingly, the adult mice used in these and subsequent experiments reported in this paper were castrated without regard to the day of the cycle, and after a week or more had elapsed they were brought into full heat, as determined by the vaginal smear. They were then injected for the next 8 days with Theelin⁷ which had been dissolved in Mazola oil, the dilutions being such that

⁶ All Theelin and Amniotin extracts used in these and subsequent experiments reported in this paper were assayed on adult castrated rats. The rat unit is based upon a 50 per cent response in twenty rats each injected three times at 12-hour intervals.

⁷ We are indebted to Parke, Davis & Co. for the crystalline oestrogenic hormone (Theelin).

the daily dose was 0.1 cc. regardless of the amount of hormone being given. By following this method of dilution and injection, we attempted to make constant the amount of oil injected, thus avoiding as far as possible differences in absorption due to different amounts of oil. The total dosages given over the 8-day period varied from 0.025 r.u. to 1.0 r.u. (table 2). Daily vaginal smears were made during the experiment and necropsy was performed on the day after the last injection.

From the perusal of table 2 it is seen at once that as little as 0.2 r.u. injected over a period of 8 days immediately following oestrus produced unmistakable mucification in some

TABLE 2
Adult castrated mice injected with Theelin

HISTOLOGICAL FINDINGS IN VAGINA AT AUTOPSY	OBSERVATIONS AND TOTAL DOSAGE IN RAT UNITS	AVERAGE DOSE IN RAT UNITS
Cornification with mucified cells in lumen, ¹ or cornification	1.0; 1.0; 1.0; 1.0; 0.5	0.9
+++ mucification or +++ mucification with some stratification beneath	1.0; 0.5; 0.5	0.66
++ mucification	0.5; 0.5; 0.4	0.46
+ mucification	0.5; 0.4; 0.4; 0.3; 0.25; 0.2	0.34
0 mucification	0.4; 0.1; 0.05; 0.025; 0; 0	

¹ None of this group desquamated many scales during the period of injections as far as could be determined by examination of vaginal smears. The doses, therefore, did not bring any of the animals into heat.

cases, and that by increasing the dose to 0.4 or 0.5 r.u. mucification equivalent to that found during the latter part of pregnancy was obtained. If the dose was further increased to 0.5 to 1.0 r.u., then typical stratification and even cornification was obtained. When the total dose was 0.1 r.u. or less, practically no mucification was produced and the vagina closely resembled that of a 9-day castrate.⁸ The plus signs recorded

⁸ In castrated animals the vaginal epithelium is one or two cells thick and has the appearance of being mucified. However, the superficial layer of cells is more columnar than mucified and we have considered such a vaginal epithelium as 0 (fig. 14). We do not consider the vagina to be mucified unless it equals that designated by +.

in table 2 refer to the degree of mucification present. These degrees of mucification are illustrated in figures 11, 12, and 13, and, while they are not from this series of animals (with the exception of 6 and B-12), they are accurate representations of the several degrees of mucification produced and we wish to use them as definite standards. In fact, all degrees of mucification in mice described by plus signs in this paper refer to the use of these sections as standards. It is of interest that one may build up a very thick stratified epithelium resembling the epithelium of oestrus (fig. 9) without the appearance of a typical heat smear, and that good mucification may come to lie on a stratified epithelium (fig. 10). Good mucification is produced, therefore, in the castrated mouse by the injection of from 0.2 to 0.5 r.u. of Theelin for the first 8 days following heat. If the amount is increased from 0.5 to 1.0 r.u. stratification and cornification is usually produced.

PRODUCTION OF MUCIFIED CELLS IN THE VAGINAL EPITHELIUM
OF CASTRATED ADULT AND NON-CASTRATED
IMMATURE GUINEA-PIGS

In 1924, Courrier reported the production of mucified cells in the vaginal epithelium of adult guinea-pigs which had been castrated and injected with follicular fluid, the oestrin content of which was not determined. In view of the great progress in assay and purification of oestrogenic substances in recent years, it seemed of value to repeat the experiments of Courrier and thus determine whether the mucification reaction could be produced in a species other than the mouse by purified oestrin (Amniotin and Theelin).⁹

In the experiments about to be described, five adult virgin female guinea-pigs were used. They were castrated by the flank approach in the usual manner and then injected for varying periods of time with varying amounts of either Amniotin or Theelin. The hormone was dissolved in either water or 10 per cent alcohol. Injections were made daily over a period of from 20 to 68 days and the reaction of the

⁹ We are indebted to E. R. Squibb & Sons for the Amniotin used in these experiments.

vagina was followed by daily vaginal smears and repeated biopsies of the vaginal epithelium. Altogether in these five animals thirty-two biopsies were performed. Since this small number of animals was studied in such detail, the protocols of each are abstracted below.

Guinea-pig A-1. Castrated in heat and then given 5 r.u. Amniotin per day for the next 5 days. During this period the smear consisted of white cells and cornified cells. Biopsy of the vagina on the fifth day showed the vaginal epithelium to be thick and moderately cornified. The vagina remained open. For the next 6 days the dose was reduced to 2.5 r.u. per day and the vaginal smears still contained many cornified cells. Injections were therefore discontinued for 2 days. For the next 25 days 0.5 r.u. of Amniotin per day was given and biopsies of the vagina were made on the fourth, seventh, twelfth, twenty-first, and twenty-ninth days. Those samples removed on the fourth and seventh days showed a low epithelium without cornification or mucification. The one removed on the twelfth day showed a fairly thick epithelium with some mucification of the outer cells, and the one removed at the twenty-first day showed excellent mucification. The specimen obtained on the twenty-ninth day showed that the mucification had largely disappeared, the epithelium now being thick, non-mucified, and with the superficial layers verging on cornification. Autopsy, 24 days after stopping the injections, revealed that the ovaries had been removed completely.

Guinea-pig A-6. Castrated in heat. No injections for 4 days. She was then injected with 0.5 r.u. of Amniotin per day for 32 days and the vagina was biopsied on the fourth, seventh, tenth, fifteenth, twenty-fourth, and thirty-second days. By the fifteenth day fairly good mucification was obtained. This was still present at the thirty-second day, but it was not quite as well developed as that in no. A-1. During the next 10 days the dose of Amniotin was raised to 1.5 r.u. per day, and tissue removed by biopsy at the end of this period showed a thick squamous epithelium without mucifica-

tion or cornification. Autopsy some time later revealed no ovarian tissue.

Guinea-pig A-5. Castrated. Treated the same as no. A-1, except that autopsy was performed on the twenty-eighth day. Injection of 0.5 r.u. Amniotin per day for 28 days produced excellent mucification by the twenty-first day (fig. 2). Autopsy showed that the ovaries had been excised completely.

Guinea-pig A-8. Castrated in heat. She was then injected with 0.5 r.u. of Amniotin per day for the next 68 days with biopsies on the fifth, fourteenth, twenty-second, thirty-second, forty-sixth, sixtieth, and sixty-eighth days. These specimens showed a gradual increase of mucification, so that by the thirty-second day it was excellent, although not as well developed as that seen in late pregnancy (fig. 1). This remained at the same stage of development for the remainder of the period. At no time during the 68 days of injection was there any relaxation of the symphysis or secretion of milk.

Guinea-pig A-4. Castrated 6 days after oestrus and a sample of the vaginal mucosa removed at the same time. She was then injected with 0.5 r.u. of Theelin per day for the next 20 days and biopsies of the vagina were made on the twelfth and twentieth days. The specimen removed at the time of castration showed low epithelium without mucification or cornification and the one removed on the twentieth day showed excellent mucification.

Guinea-pig A-3. Castrated about 24 hours before oestrus (deduced from the fact that the ovaries contained large follicles and the vagina showed mucification). Biopsies of the vagina were made on the sixth, thirteenth, and twenty-first days after castration to obtain sections of the castrate vagina (fig. 4). She was then allowed to go for 8 more days, making her a 29-day castrate. During this entire period the vagina remained closed, except for the days on which biopsies were made. Beginning on the twenty-ninth day of castration, and for a period of 5 days she was given 5 r.u. of Theelin daily. The vagina opened on the day of the fifth injection and the smear consisted of mucified cells. The specimen removed

on the sixth day showed a thick stratified squamous epithelium, the superficial layers of which showed typical cornification (fig. 3).

These experiments show that by the continued injection of 0.5 r.u. of either Amniotin or Theelin into adult, castrated, female guinea-pigs, vaginal mucification is produced which is indistinguishable from that found during normal pregnancy. Larger injections of oestrin (5.0 r.u. per day) produce stratification and cornification.

In view of the results obtained with oestrin in the adult castrated guinea-pigs, somewhat similar experiments were carried out in non-castrated immature female guinea-pigs to determine whether the mucification reaction was dependent upon the animal attaining puberty. The exact age of all immature animals subsequently described was known, but this will not be given for each animal. Suffice it to say that the initial injection was made some time within the first 7 days of life in all cases, at a time when the vaginal outlet was not yet open, and long before any signs of puberty were apparent in the genital tract. The animals were injected subcutaneously twice daily with the desired amount of oestrin. The oestrin was stored in 20 per cent alcohol and diluted with an equal amount of water immediately before using. Thirteen animals were used, three being kept as controls to establish the normal state for untreated immature guinea-pigs.

The first group consisted of four animals (A-10, A-12, A-15, A-16), each of which was injected with from 2 to 5 r.u. of oestrin daily for 4 days with autopsy on the fourth or fifth days (table 3). One (A-10) died on the day after the last injection and before the vagina opened. Sections from A-10 (fig. 6) showed the vagina to be of approximately adult size and the epithelium to be well mucified and almost indistinguishable from the vagina of a mid-term pregnancy. There was no semblance of stratification of the basal layers. The other three animals were killed on the fourth or fifth day and after the vagina had opened. The opening was preceded by hyperemia and swelling of the vulva. The first smear

after opening consisted of mucified cells, leukocytes, and no scales. Sections of the vagina (fig. 7) showed the epithelium

TABLE 3
Immature guinea-pigs treated with oestrin

GUINEA-PIG NO.	DAILY DOSAGE OF OESTRIN IN RAT UNITS ¹	NUMBER OF DAYS INJECTED	TOTAL AMOUNT OF OESTRIN INJECTED RAT UNITS	DAY VAGINA OPENED	DAY OF AUTOPSY ²	RESULTS OBTAINED IN THE VAGINA AT AUTOPSY
A-10	5	4	20	Closed	5	Good mucification. No stratification. Figure 6
A-12	5	4	20	4	4	Good mucification with stratification beneath. Figure 7
A-15	5	4	20	5	5	Same as A-12
A-16	2	4	8	5	5	Same as A-12
A-17	2	5	10	6	6	Cornification with almost complete desquamation of mucified cells
A-11	5	7	35	4	8	Thick epithelium, non stratified, some mucification
A-13	25	9	225	4	10	Same as A-11, except for more mucification
A-14	25	9	225	4	10	Same as A-13
A-54 {	20	8	160	4	13	Thick epithelium, non-stratified, without mucification
	40	4	160			
A-53	20	15	300	5	16	Good mucification. No stratification
A-50	0	0	0	Closed	Birth	Same as A-51
A-51	0	0	0	Closed	Birth	Epithelium but two cells thick. Outer row has pale cytoplasm. Figure 8
A-52	0	0	0	Closed	9th after birth	Same as A-51

¹ A-10, A-11, A-12, A-13, and A-14 were injected with Theelin supplied by Parke, Davis & Co. A-15, A-16, A-17, A-53, and A-54 were injected with Amniotin supplied by E. R. Squibb & Co.

² The day of the first injection was counted as the first day in computations involving time.

to be radically different from that of A-10. The superficial layer was the same, i.e., well mucified, but beneath the mucified layer there was a thick, stratified epithelium the more super-

ficial layers of which were cornified in some regions. In those places where the cornification was present the superimposed mucified layer was largely desquamated, thus explaining the presence of mucified cells in the smear. Another animal (A-17) was injected for 5 days with 2 r.u. per day with autopsy on the sixth day. The vagina opened on the sixth day. Sections of the vagina showed the lumen to be filled with mucified cells. The epithelium was thick and stratified, but the mucified cells were almost completely desquamated.

The next group of five animals consists of those in which the period of injections was prolonged to 7 to 15 days. These animals were injected with from 5 to 25 r.u. of oestrin daily and, regardless of the dose, their vaginae opened on the fourth or fifth day. The initial smears were always made up almost completely of mucified cells, so that we may assume that mucification similar to that seen in the first four animals was induced by the injections. The smears on the day after opening consisted of cornified cells, mucified cells, and leukocytes, but after that, even though the same dosage was continued, the smears contained only leukocytes and a few nucleated epithelial cells. One may deduce from the smears, therefore, that the vaginal state was not the same as that found at the time of opening (mucified) or that found the day after (cornified). Histological sections of the vaginae of these animals support this deduction. One animal (A-11), autopsied on the eighth day, showed a non-stratified epithelium, but one which was several layers thick and which had cells with large vesicular nuclei and pale cytoplasm. This type of cell we have previously found in the adult animal to be typical of the premucified condition. A-13 and A-14 were given 25 r.u. per day and were autopsied on the tenth day, after receiving 225 r.u. The sections from these were essentially the same as those from A-11, except for definite mucification of the outer cells. A-53 was given 20 r.u. per day for 15 days with autopsy on the sixteenth day. In this animal mucification was produced which was similar to that seen in A-10, i.e., complete mucifica-

tion of the epithelium with but one basal layer non-mucified. It should be said, however, that the mucification was somewhat atypical, and that it resembled that produced in the adult guinea-pigs by large doses of oestrin. The last animal of this series (A-54) was given 20 r.u. per day for 8 days. The vagina opened on the fourth day and she was out of heat by the sixth day. She was then given twice the previous dose, i.e., 40 r.u. per day for 4 days, as a result of which she came into full heat (cornified cells) on the twelfth day of injections (fourth day of increased dose). Sections of the vagina obtained at autopsy on the thirteenth day showed a thick, somewhat stratified epithelium, without mucification.

The ovaries of all these immature guinea-pigs were unaffected by the treatment. Sections showed numerous small follicles some of which were undergoing atresia, but no corpora lutea or luteinized follicles. The uteri were all enlarged and of pink color and those treated for the longer periods were of approximately adult size. We see, therefore, that the oestrin used not only produced the vaginal changes mentioned above, but it also brought the uterus into the adult state without causing growth of the ovaries.

The third group of animals consisted of three which were untreated. Two (A-50, A-51) were born dead and a third (A-52) was autopsied on the ninth day of life. The vaginal outlets were not open and sections showed the vaginal epithelium to be devoid of crypts and only two cells in thickness (fig. 8). The outer row of cells had pale cytoplasm and large nuclei, but these cells are quite different from the mucified ones produced by the injection of oestrin. A glance at figures 6 and 8 makes the difference very obvious.

These observations show that injection of 5 to 25 r.u. of Theelin or Amniotin per day into newborn, non-castrated female guinea-pigs causes the vagina to open on the fourth or fifth day. The vaginal smear at opening consists of numerous mucified cells and some leukocytes. Section of the vagina at this time shows the mucosa to be made up of a superficial, desquamating, mucified layer, and a deeper stratified squa-

mous layer the more superficial cells of which are cornified. If autopsy is performed about 12 hours before the vagina opens the vaginal epithelium is well mucified and indistinguishable from that of midpregnancy. If the same daily dose of oestrin is continued after the vagina opens, the animal does not remain in heat and the vaginal epithelium again becomes mucified, but in this case it is slightly atypical.

DISCUSSION

The experiments which we have described confirm and amplify those of Robson and Wiesner ('31) and further show that mucification of the vagina can be produced by Theelin, the purified oestrogenic hormone, if proper dosages are used. We cannot deny the possibility that mucification may be due to a separate hormone distinct from Theelin, but if this be so, the Theelin supplied us is contaminated. It would seem necessary, therefore, to prove that Theelin contains more than one active principle before the existence of a special mucifying hormone can be demonstrated beyond reasonable doubt.

Harris and Newman ('31) have suggested that the production of mucification of the vagina of the non-castrated adult mouse may be used for the assay of corpus luteum extracts containing progestin (or a substance which maintains pregnancy in the castrated rat). The reliability of such a test seems doubtful, since the mucification produced is not a direct measure of the progestin present, but rather a measure of the oestrin elaborated by the animal's own ovaries plus that present in the extract injected. He (Harris, '32) is unable to produce mucification by the injection of Theelin alone in the non-castrated animal, so he maintains that his original test is reliable. His ability to produce mucification with corpus luteum extracts and not with Theelin is readily explained; in the former case it may be assumed that the extracts inhibited the cycle and thus gave adequate time for the development of mucification, whereas in the latter case the cycle was not inhibited and hence no mucification could be developed either by the animal's own ovaries or by the Theelin injected. If,

therefore, an extract should contain any substance whatsoever which inhibited the cycle, then mucification should be produced, and this in fact is so, since Hisaw, Meyer, and Weichert ('28) have demonstrated that mucification of the vagina of the non-castrated rat results when the cycles are inhibited by active corpus luteum extracts, and one of us (Meyer, unpublished data) has found a similar mucification when the cycles are inhibited by extracts of muscle and testis prepared by the same method.

Recently Fevold, Hisaw, and Leonard ('32)¹⁰ have published evidence from which they conclude that the substance responsible for the production of mucified cells in the vagina of the rat is a specific hormone of the corpus luteum, not identical with progesterin, relaxin, or oestrin. The results obtained by us and presented in this paper make it seem probable that mucification does not result from the action of a substance secreted specifically by the corpus luteum. This conclusion would not be tenable if there were not ample evidence to show that Theelin is a single chemical entity. It may be, however, that more than one substance is capable of producing mucification, but this is improbable.

In the previous part of this discussion we have stated that oestrin is the hormone directly responsible for mucification and that progesterin does not produce such a reaction. This opinion is fully substantiated by the fact that corpus luteum extracts in which progesterin has been inactivated by treatment with alkali will produce mucification, and by our own investigations as a result of which we have been able to separate oestrin from progesterin and show without destruction of either hormone that the mucifying factor is always found in a much higher concentration in the oestrin fraction. We could draw attention, however, to certain of our own data which indicate that progesterin or some other substance contained in the pro-

¹⁰ Dr. F. L. Hisaw has stated in a personal communication to us that the fraction which they had considered to contain a mucifying hormone also contained some oestrin and that the mucification obtained in the castrated rat by this fraction was probably produced by the small amounts of oestrin.

gestin fraction may reduce the ability of oestrin to produce oestrus as determined by the vaginal smears. Comparison of those animals injected with corpus luteum extracts (table 1) with those injected with Theelin (table 2) shows that in the former mucification could be produced with a wide range of dosage, whereas in the latter it could be produced with only a narrow range of dosage. This we interpret as indicating that the progestin (or some other substance) in these experiments exercises an inhibiting effect on the ability of the oestrin to produce cornification. The oestrin which has been separated from progestin by our procedure behaves the same as Theelin in this respect; it produces mucification over a very limited range of dosage, whereas the progestin which has been largely freed of oestrin produces no mucification unless very large doses are given. This interesting evidence of interrelationship in no way weakens the evidence that mucification can be produced by Theelin. It merely suggests that progestin (or some other substance) when present may alter the response to oestrin.

CONCLUSIONS

1. Extracts of the corpus luteum which contain both oestrin and progestin will produce mucification of the vaginal epithelium in the castrated mouse.
2. Mucification of the vaginal epithelium of castrated adult mice and guinea-pigs and non-castrated immature guinea-pigs can be produced by the injection of subcornifying amounts of oestrin (Amniotin and Theelin). The injection of oestrin above the optimum amount necessary to produce maximum mucification causes the mucified cells to be desquamated and replaced by stratified or cornified cells.

LITERATURE CITED

- ALLEN, W. M. 1932 The preparation of purified progestin. *J. Biol. Chem.*, vol. 98, p. 591.
- ALLEN, W. M., AND R. K. MEYER 1932 The preparation of oestrin-free progestin from corpora lutea of swine. *Anat. Rec.*, vol. 54, Supplement, p. 26.
- CLAUBERG, C. 1930 Experimentelle Untersuchungen zur Frage eines Mäusetestes für das Hormon das Corpus luteum. *Zent. f. Gynäk.*, Bd. 54, S. 1154.

- CORNER, G. W., AND W. M. ALLEN 1929 Physiology of the corpus luteum. II. Production of a special uterine reaction (progestational proliferation) by extracts of the corpus luteum. *Am. J. Physiol.*, vol. 88, p. 326.
- COURRIER, R. 1924 Le cycle sexual chez la femelle des Mammifères. Étude de la phase folliculaire. *Arch. de Biol.*, T. 34, p. 370.
- FELS, E. 1931 Zur Frage des Corpus luteum Hormons und seines spezifischen Testes. *Zent. f. Gynäk.*, Bd. 55, S. 514.
- FEVOLD, H. L., F. L. HISAW, AND S. L. LEONARD 1932 Hormones of the corpus luteum. The separation and purification of three active substances. *J. Am. Chem. Soc.*, vol. 54, p. 254.
- FEVOLD, H. L., F. L. HISAW, AND R. K. MEYER 1930 Purification of the hormone of corpus luteum responsible for progestational development and other reactions. *Proc. Soc. Exp. Biol. Med.*, vol. 27, p. 606.
- HARRIS, R. G. 1932 Mucification of the vaginal epithelium of mice as a test for pregnancy-maintaining potency of extract of corpora lutea. *Science*, vol. 76, p. 408.
- HARRIS, R. G., AND D. M. NEWMAN 1931 A practical test for potency of extracts of corpora lutea. *Science*, vol. 74, p. 182.
- HISAW, F. L., R. K. MEYER, AND C. K. WEICHERT 1928 Inhibition of ovulation and associated histological changes. *Proc. Soc. Exp. Biol. Med.*, vol. 25, p. 754.
- LATASTE, F. 1892 Transformation périodique de l'épithélium du vagin des rongeurs (rythme vaginal). *C. R. Soc. Biol.*, T. 44, p. 765.
- LEONARD, S. L., F. L. HISAW, AND H. L. FEVOLD 1932 Further studies of the follicular-corpora luteum hormone relationship in the rabbit. *Am. J. Physiol.*, vol. 100, p. 111.
- LONG, J. A., AND H. M. EVANS 1922 The oestrous cycle in the rat and its associated phenomena. *Memoirs of the Univ. of Calif.*, vol. 6.
- MEYER, R. K., AND W. M. ALLEN 1932 The production of mucification of the vaginal epithelium of rodents by the oestrous hormone. *Science*, vol. 75, p. 111.
- REITTERER, E. 1892 Évolution de l'épithélium du vagin. (Deuxième note). *Memoires Comptes Rendus Societé de Biologie*, T. 44, p. 566.
- ROBSON, J. M. 1931 Mucification in the mature mouse caused by oestrin. *J. Physiol.*, vol. 71, p. III of Proceedings of the Physiological Society.
- ROBSON, J. M., AND B. P. WIESNER 1931 Causation of mucification and cornification in the vagina of the mouse. *Quart. J. Exp. Physiol.*, vol. 21, p. 217.
- SELLE, R. M. 1922 Changes in the vaginal epithelium of the guinea-pig during the oestrous cycle. *Am. J. Anat.*, vol. 30, p. 429.
- WIESNER, B. P., AND J. S. PATEL 1929 The Beta hormone. *Nature*, vol. 123, p. 449.

PLATE 1

EXPLANATION OF FIGURES

1 Photomicrograph from a section of vagina obtained by biopsy 24 hours antepartum during normal pregnancy from guinea-pig A-27. $\times 155$.

2 Photomicrograph from a section of vagina obtained by biopsy from guinea-pig A-5. This pig was castrated and then given 0.5 rat unit of Amniotin per day for a period of 21 days immediately preceding this biopsy. Compare this histological picture with that seen in figures 1, 4, and 5. $\times 155$.

3 Photomicrograph from a section of vagina obtained by biopsy from guinea-pig A-3, showing cornification produced by the injection of 5 r.u. of Theelin per day for 5 days into a 29-day castrate. Compare with figure 4, a section from the same pig 21 days after castration and before the injections of Theelin were begun. $\times 155$.

4 Photomicrograph of a biopsy section of vagina from guinea-pig A-3, a 21-day castrate. $\times 155$.

5 Photomicrograph of a biopsy section of vagina obtained from guinea-pig A-3, 12 to 24 hours before oestrus. Note that beneath the mucification there is considerable growth of stratified epithelium. In other parts of the section there were signs of early cornification. $\times 155$.

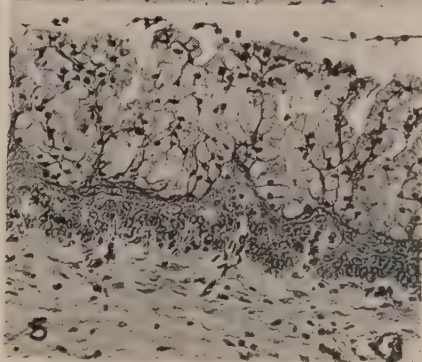
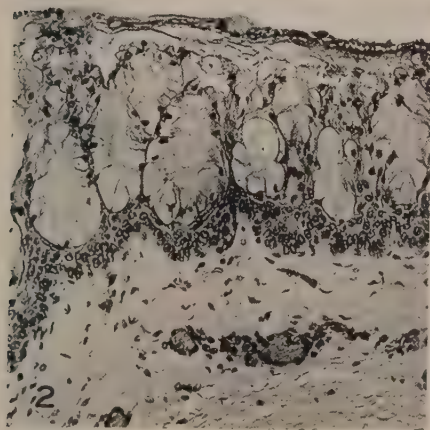
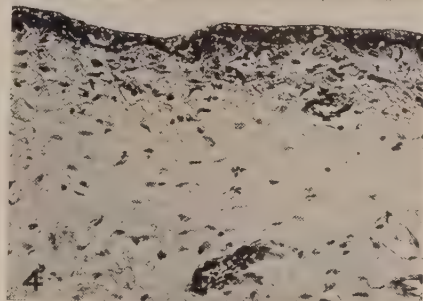
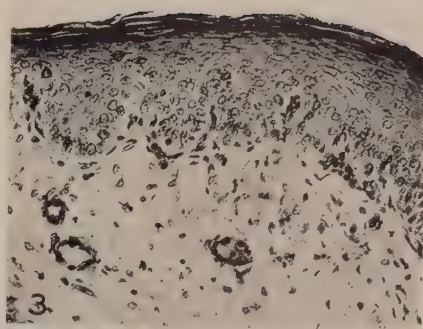


PLATE 2

EXPLANATION OF FIGURES

6 Photomicrograph of a section from the vagina of immature guinea-pig A-10. This degree of mucification was produced by 5 r.u. of Theelin per day for the first 4 days after birth with autopsy on the fourth day. The vagina was not open. Compare with figure 8, an untreated litter mate. $\times 155$.

7 Photomicrograph of a section from the vagina of immature guinea-pig A-12. This animal received 5 r.u. of Theelin per day for the first 4 days after birth with autopsy on the fifth day. The vagina opened on the fifth day and the smear consisted of mucified cells only. The mucified layer is quite similar to that seen in figure 6, but beneath the mucified cells there is a layer of stratified epithelium which in some regions is undergoing slight cornification causing desquamation of the mucified cells. $\times 155$.

8 Photomicrograph of a section from the vagina of newborn guinea-pig A-51. Autopsy was performed immediately after birth. The epithelium consists of two rows of cells, the outer of which is composed of columnar cells with pale cytoplasm. Contrast this with figures 6 and 7. $\times 155$.

9 Photomicrograph of a section from the vagina of mouse B-24. This animal was castrated, brought into heat with Theelin, and then injected with a 0.5 N sodium hydroxide soluble fraction of a corpus luteum extract for 8 days. There is excellent stratification of the epithelium without cornification. $\times 155$.

10 Photomicrograph of a section from the vagina of mouse no. 6. This animal was castrated in heat and then injected with 0.0625 r.u. of Theelin per day (i.e., 0.5 r.u. total) for 8 days, with autopsy on the day following the last injection. The superficial layer of epithelium is well mucified and the deeper layer is stratified. This condition is similar to that found in the immature guinea-pig under similar conditions and illustrated in figure 7. If the injections had been continued for another day the mucified layer would probably have desquamated. $\times 155$.

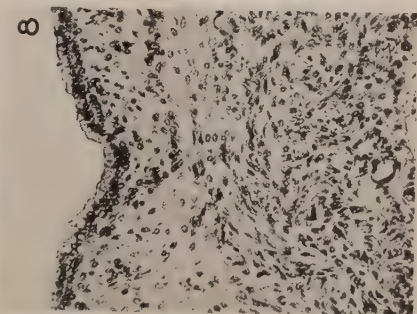
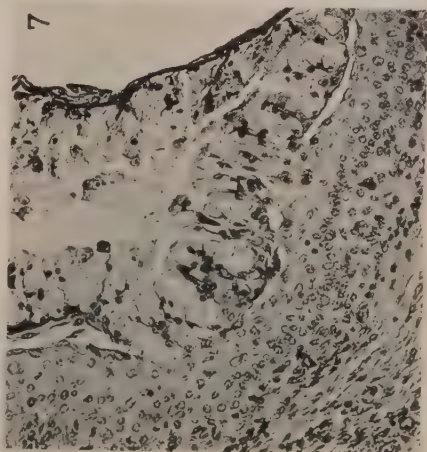
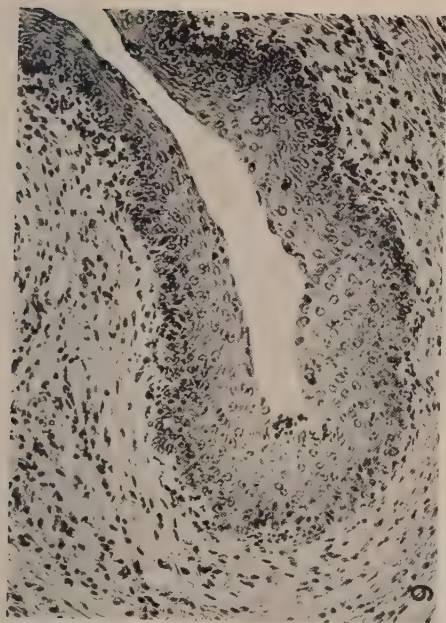
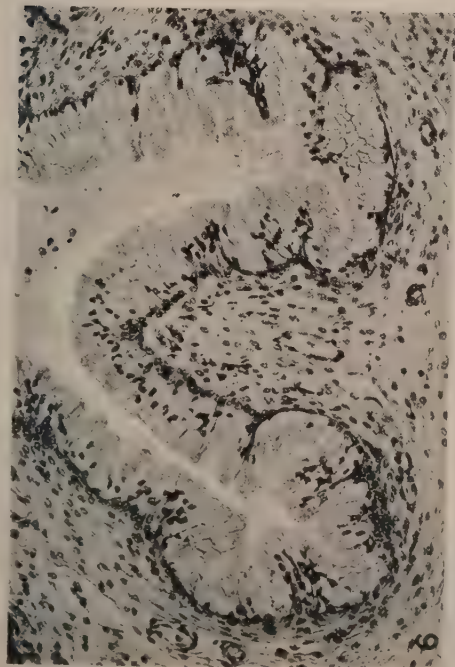


PLATE 3

EXPLANATION OF FIGURES

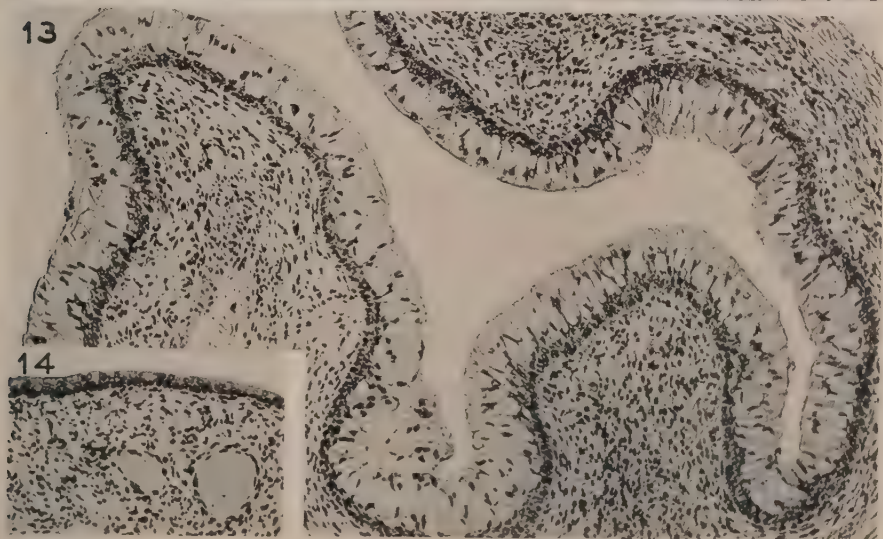
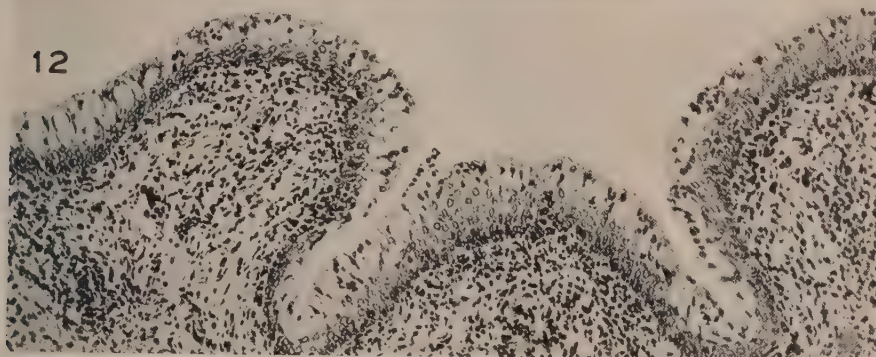
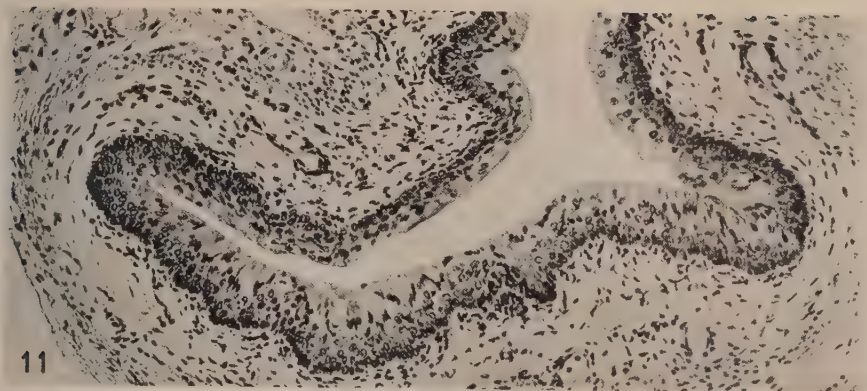
11 Photomicrograph from a section of the vagina of mouse B-12. This animal was castrated, brought into heat with Theelin, and then injected for 8 days with a total of 0.3 r.u. of Theelin. This represents a + mucification. $\times 155$.

12 Photomicrograph from a section of the vagina of mouse B-27. This animal was castrated, brought into heat with Theelin, and then injected for 8 days with corpus luteum extract no. 89 containing 0.33 rabbit unit of progesterin. The mucification produced is due to the oestrin which the extract contains and not to the progesterin. This represents a ++ mucification. $\times 155$.

13 Photomicrograph from a section of the vagina of mouse B-28. This animal was castrated, brought into heat with Theelin, and then injected for 8 days with corpus luteum extract no. 88 containing 1.33 rabbit units of progesterin. The mucification, as in the case of figure 12, is due to the oestrin present in the extract. This is a +++ mucification. $\times 155$.

14 Photomicrograph of a section of the vagina from mouse B-21. This animal was castrated, brought into heat with Theelin, and then after no further treatment was sacrificed on the ninth day after the induced oestrus. This section serves as a control for those represented in figures 9, 10, 11, 12, and 13.

Figures 11, 12, 13, 10, and 9 represent the changes which may be produced by gradually increasing the amount of Theelin from 0.3 r.u. to 1.0 r.u. total in 8 days.



MILK-PRODUCTION CURVE OF ALBINO MICE

E. V. ENZMANN

Laboratory of General Physiology, Harvard University, Cambridge, Massachusetts

SIX FIGURES

I

The dependence of the growth of the suckling mouse upon the milk supply of the mother was first shown by MacDowell and his collaborators ('30). The object of the present study was to bring to light the quantitative relationship between the amount of food eaten and the growth of the suckling mouse. To study this relationship it was necessary to examine the milk-producing capacity of lactating mothers; this was done by constructing the 'milk curve' of the mouse.

Although there are many papers dealing with the initiation of milk secretion in the mouse (compare Enzmann and Pincus, '33; Turner, '32), no indications could be found in the pertinent literature that the milk-secreting capacity of the mouse during lactation has been made a subject of close study. Most of the work on milk secretion has been done on human subjects and on domestic animals whose milk is used in human nutrition.

Holt and Howland ('22) gave figures expressing the quantity of milk secreted by human mothers from parturition until the end of the tenth month. Brody, Ragsdale, and Turner ('23-'24) studied the milk curve of the cow. Thew showed that milk secretion rises for a time after parturition, reaches a peak, and then declines exponentially. The rising and the falling parts of the milk curve may be represented by a curve of a first order reaction. Gaines and Davidson ('26) found that this relation is followed even more closely if the energy yield of the milk is taken into account. Gowen ('20) studied the milk yield of the cow in relation to the age of the animal. Weatherford ('29), using the amount of Golgi material as

criterion, finds that in the rat the secretory activity reaches a maximum around the tenth day. Turner ('32) made whole-mounts of the mammary glands of the mouse which confirmed Weatherford's observations.

Observations on the mouse as well as on other mammals tend to show that milk flow is influenced by a large number of factors: race, weight and age of the mother, nature of the food, number of offspring, rate of emptying the mammary glands, and others.

II

In human mothers, milk is often obtained by means of the breast-pump. Experience has shown, however (compare Holt and Howland, '22), that the total milk production can be estimated much more accurately by weighing the infant before and after feeding. In the present experiments a similar method was employed.

In the first set of experiments healthy animals of approximately the same age and weight, and of the same strain, were used as in earlier observations on lactation and pregnancy (Enzmann and Pincus, '33; Enzmann, Saphir, and Pincus, '32). The litter size varied from 4 to 13 young. In a second set of experiments the same number of mothers was used, but the litter size was kept constant at four nursed young in each litter.

The litter was taken from the mother immediately after birth, before the young had their first feeding. Half of the young were returned to their mother after their weights had been determined; the rest of the young were kept in an empty milk tin half filled with absorbent cotton to prevent excessive loss of heat. It was found that the young kept apart from the mother retained in every case a higher body temperature than the set of young left with the mother. Using paper shavings instead of cotton gave better results.

In the preliminary experiments the two sets changed places every 2 hours, but later shifts of 6, and in a few of 12, hours were made. The reason for increasing the time between shifts was that the lactating mothers do not keep a regular feeding schedule; in the 2-hour shifts it often happened that the young with the mothers had not been fed at all, while the next set of young received higher rations. Consequently, one set grew faster, which introduced a new complication, since heavier animals, as a rule, exact a larger share of milk during their turn of feeding. The 6-hour shifts gave the best results, but proved somewhat fatiguing on the experimenter.

In each experiment the following observations were recorded: weight of each set of animals before and after feeding, and the amount of dry food consumed by each set when the young were kept apart from the mother. From the losses of weight between feedings one may calculate the amount of food which has been built into body substance and the amount of loss in feces, urine, heat, and other losses, on the assumption that the losses are the same whether the young are with the mother or separated from her. From the observations on the consumption of dry food during the time when the young are away from the mother one may similarly estimate the total solid food that would have been consumed.

There are sources of error in this procedure, due chiefly to the fact that the young will eat but little dry food as long as they have a chance to suckle. The dry food consisted of accurately weighed pieces of commercial 'Dog Chow' which were weighed at every shift. Losses of food due to crumbs are small if the pieces chosen are round and just large enough so that the animals can easily hold them between their forepaws. Aside from certain objections to this method, there is the serious complication that litters which have been split up do not grow as well as litters which are constantly with the mother. The values expressing the daily gain of the suckling mice used in the experiments are all below those obtained from controls. A comparison of the growth curves of the animals used in the lactation experiments with control animals should give some indication of the necessary correction. This was not done, since it is not relevant to the immediate problem.

III

The variations in the daily amounts of milk flow as measured by this method are considerable; at one day the total may be 3 gm. and the next day twice this amount. Part of this variation is doubtlessly due to the choice of time units for changing the sets. The feeding times are irregular and often the change has to be made while the young are being fed.

The amount of milk produced on the day following parturition is usually small. Secretion then rises to a maximum which lies at the average around the tenth day and declines from then until the end of lactation. The weaning may be

considered complete on the twentieth day, although the young may keep on suckling for a variable period thereafter. The end-point of the milk curve differs from one mother to another. The daily milk production of mothers with litters of varying sizes (4 to 13 young) is shown in table 1 and in figure 1. Each full line in the figure represents the milk

TABLE 1
Daily milk production of mothers with litters, ranging from 4 to 13 young per litter. (Weight in grams)

DAYS AFTER BIRTH	NO. 60 7 YOUNG	NO. 133 10 YOUNG	NO. 147 7 YOUNG	NO. 74 6 YOUNG	NO. 51 4 YOUNG	NO. 36 13 YOUNG	NO. 53 6 YOUNG	NO. 225 8 YOUNG	MEANS
1	0.15	4.37	0.80	1.41	0.62	0.05	2.11	1.27	1.347
2	2.00	7.57	1.32	1.55	1.36	0.19	2.32	2.17	2.310
3	2.28	5.16	2.16	2.21	1.86	5.48	2.42	3.07	3.080
4	2.82	3.74	2.34	2.10	1.64	6.95	3.62	3.01	3.277
5	3.38	3.22	3.02	2.84	2.11	6.79	3.94	3.68	3.622
6	3.08	4.76	2.91	3.03	1.95	4.33	4.25	3.62	3.491
7	3.49	4.50	2.75	2.95	2.77	4.72	5.16	3.60	3.742
8	6.39	3.08	4.24	3.47	2.84	5.38	4.33	3.91	4.205
9	3.07	4.36	3.97	2.88	2.48	7.39	4.58	4.18	4.114
10	2.80	5.07	3.52	2.22	3.02	7.86	4.10	3.84	4.054
11	4.51	5.56	3.08	2.52	3.22	6.20	3.40	3.88	4.045
12	2.70	4.17	3.90	2.93	3.06	5.02	3.40	3.58	3.595
13	2.65	7.02	3.22	3.02	2.85	3.62	3.73	3.91	3.752
14	4.25	4.88	2.47	3.00	3.14	2.84	3.50	3.72	3.475
15	4.12	3.91	3.02	2.90	3.07	2.50	3.24	2.62	3.172
16	3.22	4.00	3.52	2.61	2.75	4.16	1.47	2.22	2.994
17	3.38	3.86	2.82	1.92	2.16	4.26	1.68	2.40	2.810
18	3.10	4.55	2.68	1.85	1.49	5.40	0.82	2.29	2.797
19	2.82	4.42	2.70	2.08	1.31	4.70	0.92	1.62	2.574
20	3.18	4.34	2.52	1.94	1.12	4.03	0.84	1.52	2.436
21	3.03	4.02	2.34	1.55	1.04	4.09	0.62	1.96	2.331

The underlined numbers signify that at the corresponding day one young died or was killed by the mother.

production of one mother. The results are not very satisfactory, because some young were lost during the experiment. This is indicated by underlining the numbers in table 1 at the time when the loss occurred. The daily fluctuations are considerable. The calculated averages of the daily milk production of eight mothers, however, yields a smoother curve

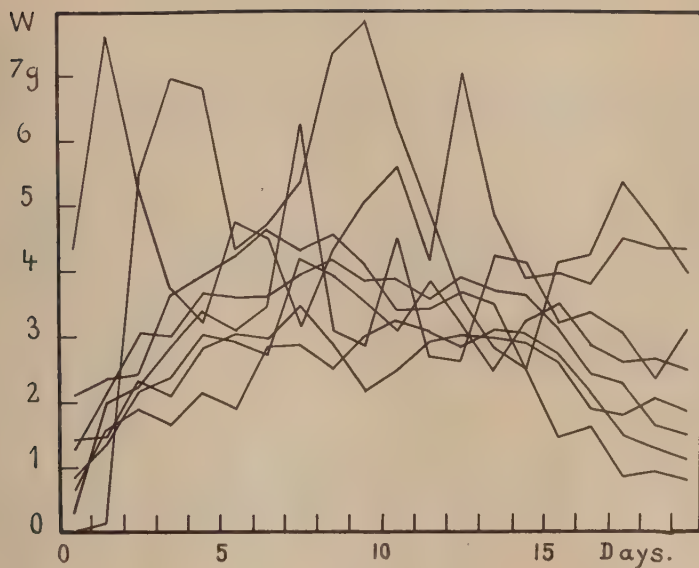


Fig.1 Milk production of mothers nursing litters of various sizes (4 to 13 young). Each line represents the milk production of one mother.

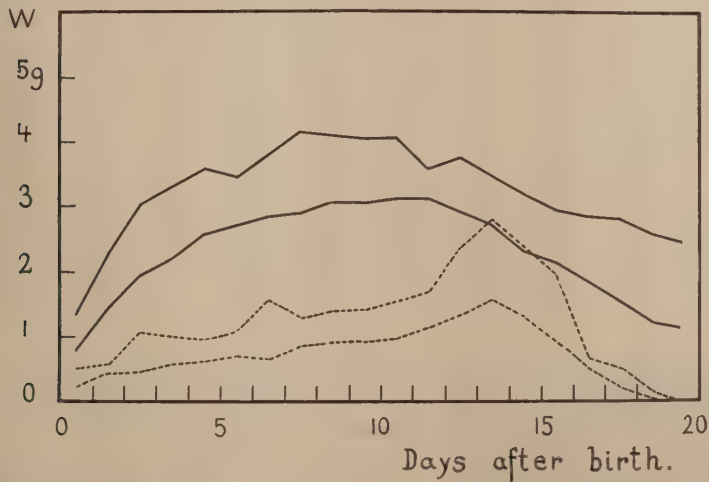


Fig.2 Average curves of albino mice. The upper full line represents the averages for mothers suckling large litters; the lower full line represents the averages for mothers suckling litters of four young. The upper dotted line represents daily losses of large litters, the lower dotted line shows the losses of litters of four.

(fig. 2). The average milk curve shows the general course of the changes in milk flow; the curve rises steadily, reaches a maximum around the tenth day, and declines steadily after that.

The daily milk production of mothers suckling litters of four young is shown in table 2 and in figure 3. Two of the litters had been reduced to four at birth, one from six young,

TABLE 2

Daily milk production of mothers suckling litters of 4 young. (Weight in grams)

DAYS AFTER BIRTH	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	NO. 7	NO. 8	MEANS
1	0.26	0.52	1.82	0.08	0.68	1.62	1.24	0.42	0.830
2	1.26	1.70	2.08	0.27	1.08	2.62	1.55	0.78	1.417
3	1.82	2.18	1.96	0.92	2.03	2.80	2.14	1.68	1.941
4	1.93	2.62	2.19	1.69	1.58	2.38	2.80	2.30	2.186
5	2.44	2.82	2.32	2.32	2.10	2.61	3.10	2.88	2.574
6	2.72	3.04	2.50	2.10	2.22	2.77	2.91	3.26	2.690
7	2.62	3.30	2.68	2.38	2.35	2.79	3.19	3.38	2.836
8	2.76	3.40	2.66	2.31	2.08	2.80	3.11	3.69	2.851
9	3.24	3.54	2.82	2.65	2.55	2.42	3.70	3.58	3.026
10	3.46	3.43	2.91	2.57	2.77	2.48	3.25	3.58	3.056
11	3.40	3.68	2.87	2.67	2.94	2.57	3.28	3.50	3.114
12	3.12	3.66	3.02	2.84	2.73	2.55	3.25	3.70	3.109
13	3.22	3.32	2.74	2.54	2.90	2.32	3.00	3.12	2.895
14	2.52	3.08	2.45	2.68	2.82	2.25	2.88	2.96	2.705
15	2.22	2.72	2.32	2.48	2.30	2.04	2.77	1.80	2.331
16	1.60	2.27	1.81	2.40	2.61	1.62	2.52	2.14	2.121
17	1.45	1.98	1.90	2.13	1.96	1.67	2.28	1.25	1.827
18	1.27	1.78	1.75	1.54	1.08	1.65	1.98	1.32	1.545
19	1.11	1.31	1.50	1.35	0.33	1.70	1.80	0.77	1.234
20	1.00	1.17	1.63	1.02	0.62	1.40	1.76	0.54	1.142
21	0.82	1.06	1.56	1.12	0.08	1.32	1.54	0.34	0.980

the other from seven young. Both mothers started out with a high milk production, but after a few days showed about the same capacity as the mothers which gave birth to four young. In these experiments the results are more uniform, although there is still a considerable difference between mothers, and variation from day to day. The average milk curve is shown in figure 2.

The daily losses of large litters are presented in table 3 and in figure 4, and those of litters of four young in table 4 and in figure 5. Here again large litters are less uniform than litters of four young. The losses rise steadily from the day of birth to the end of the second week of life and decline rapidly afterward. The decline of the losses in weight, when the young are kept apart from the mother, coincides with the change from milk to solid food. The young have their eyes

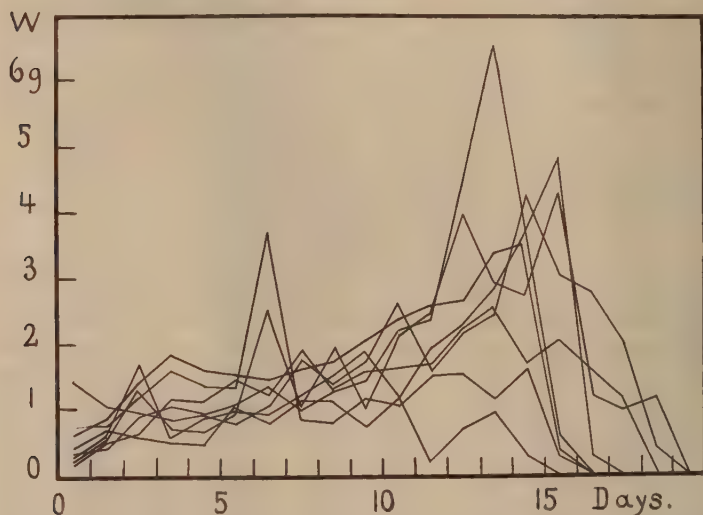


Fig. 4 Daily losses in body weight of large litters, due to defecation, urination, and other causes. The losses were calculated on the assumption that the suckling mice would lose as much when they are left with the mother as when separated from her. Each line represents the losses of one litter.

open at this time and begin to eat and drink water daily in increasing amount. The determination of the amounts of milk consumed during this period, from the end of the second week of life on, becomes less and less reliable, because the young have access to the food supply of the mother and it is difficult to estimate the amount of solid food and water taken by the young when they are with the mother. But estimating the amount of dry food the young consume while away from

TABLE 4

*Losses of whole litters; each litter consisting of 4 young. All sources combined
(Weight in grams)*

DAYS AFTER BIRTH	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	NO. 7	NO. 8	MEANS
1	0.27	0.20	0.21	0.18	0.15	0.32	0.32	0.24	0.236
2	0.34	0.36	0.31	0.52	0.22	0.26	0.64	0.52	0.396
3	0.52	0.79	0.46	0.36	0.21	0.26	0.50	0.60	0.462
4	0.57	0.70	0.42	0.50	0.27	0.78	0.52	0.60	0.545
5	0.63	1.08	0.44	0.68	0.26	0.74	0.54	0.68	0.631
6	0.51	1.08	0.48	0.75	0.60	0.60	0.80	0.81	0.703
7	0.74	1.00	0.52	0.77	0.38	0.62	0.68	0.75	0.682
8	0.62	1.12	0.82	0.67	0.48	0.91	0.50	1.33	0.806
9	0.88	0.98	0.62	0.76	0.55	1.12	0.90	1.02	0.854
10	0.76	1.18	0.68	0.88	0.58	1.00	1.10	0.79	0.871
11	0.92	1.58	1.07	0.77	0.48	1.15	1.20	0.74	0.980
12	0.80	1.36	1.02	1.16	0.83	1.47	1.90	0.98	1.196
13	1.25	1.18	1.28	1.62	0.99	1.68	1.36	1.08	1.306
14	1.44	1.74	1.45	1.58	0.78	1.50	1.48	2.58	1.569
15	1.16	2.25	0.86	1.78	0.67	1.27	1.32	1.45	1.345
16	1.00	1.48	0.72	1.17	0.48	0.80	0.88	0.70	0.904
17	0.38	0.96	0.36	0.57	0.27	0.32	0.78	0.32	0.495
18	0.26	0.61		0.18			0.55	0.18	0.222
19	0.12	0.32					0.20		0.080
20									0.000

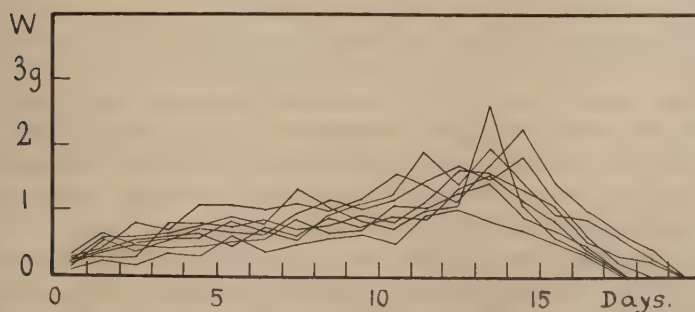


Fig. 5 Daily losses in body weight of litters of four young, due to defecation, urination, and other causes. The losses were calculated as in figure 4. Each line represents the losses of one litter.

the mother shows that this quantity increases rapidly from day to day as the young become more and more independent. An actual measure of the degree of independence of the young has been evolved; the results will be reported in a later paper. It may be said that the suckling young mice are capable of subsisting on solid food as soon as they open their eyes. Animals which have been separated from their mother at the age of 10 to 15 days starve until their eyelids part, and if the loss of weight has not been too severe they start to eat and begin to gain weight after several days.

As a rule, a mother produces more milk the more young she has to feed. This is true whether she feeds her own young or foster children. She may be made to increase her milk production by addition of young from other mothers to her own litter.

IV

The increase of milk production according to the number of young nursed is, however, not proportional to the number of young, but to some power of it. The relation is similar to that discovered by Crozier (Enzmann and Crozier, in press) for the weight increase with increasing size at birth. This is indicated in figure 2. The average litter size in the group of mothers with large litters was 6.1. The amount of milk produced by these mothers, however, is not 1.5 times as much as that elaborated by mothers suckling litters of four, as one should expect if milk secretion increased rectilinearly with litter size, but less. Toward the end of the lactation period this relation does not hold strictly, because the amount of dry food eaten is proportional to the litter size. This fact gradually modifies the power function relation.

During the time between birth and the opening of the eyes the young mice are entirely dependent upon the mother's milk. It is therefore not surprising to find that the milk curve and the growth curve of the suckling young run parallel during this period. The differential growth curve (picturing the increase in weight for each day over the weight of the preceding day) and the milk curve are shown in figure 6.

The growth curves are based on the averages of twenty males and twenty females which were used as controls. The deviations between the differential growth curve (smooth line) and the milk curve (line connecting the tops of the vertical columns) are largely due to, 1) lesser growth of the litters which were used for the milk experiments, because of the short periods of starvation while the young are kept apart from the mother and, 2) inevitable sources of error, especially during the latter part, when the young begin to eat solid food.

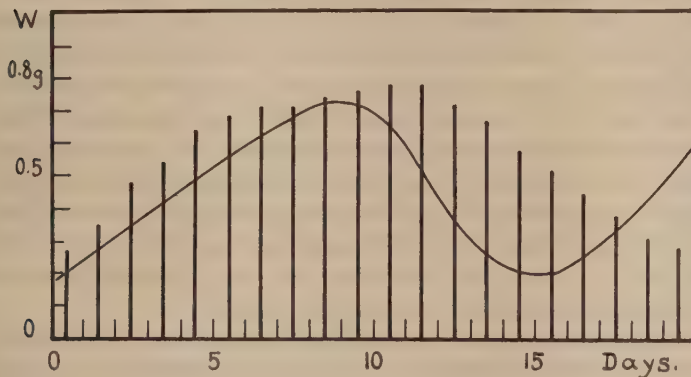


Fig. 6 Milk curve and differential growth curve for the first 20 days after birth. The smooth curve represents the smoothed average differential growth curve of 20 males and 20 females. The vertical columns represent the amount of milk measured at the corresponding times.

Earlier work on the growth curve of the mouse (Ostwald, '08; Robertson, '16, '27; Judson, '16; Mendel, '18; Gates, '25; Saller, '27; Davenport and Swingle, '27; and MacDowell, Gates, and MacDowell, '30) has shown that there is a depression of the growth rate around the fifteenth day after birth. The constant position and constant occurrence of this depression has been one of the mainstays of Robertson's theory of growth cycles. In support he cites the fact that the depression occurs regardless of whether the young are left with the nursing mother or not. Saller ('27) supports Robertson. Meanwhile strong evidence has been brought

forward by MacDowell and his co-workers ('30) to show that the depression is due chiefly to nutritional disturbances. They have shown by ingenious experiments that the growth of the suckling mouse depends at all stages during lactation upon the amount of nutrition received. In some of their experiments they succeeded in increasing the amount of milk available for each suckling young. The result was that the amount of milk did not constitute a limiting factor of growth from the tenth day on and there was no gradual decline of the growth rate, initiating the depression. Instead, the depression set in suddenly near the fifteenth day, showing that new factors become operative (compare figs. 2, 4, and 7 of their paper). The authors, however, believe that the break at the end of the second week of life is independent of the quantity of milk elaborated by the mother, for a mother may raise a new litter of young after having suckled a litter for 18 days and provide more milk for the new litter than was given to the old one. Furthermore, spaying the mothers after they have given birth to a litter does not modify the break in the growth curve of the suckling young.

The present experiments indicate that there is an actual decline in the milk production of mothers which sets in even before the young open their eyes and begin to eat solid food. This is in agreement with the experiments of Weatherford ('29) and Turner ('32) that the secretory activity in rat and mouse reaches a maximum at the tenth day. When the rate of milk secretion declines, the growth rate of the young declines; the point of maximal milk production therefore marks the beginning of the depression of growth. The present experiments cannot decide the question raised by MacDowell and his collaborators as to whether the milk flow declines because the young exact a smaller amount from the time when their eyes open, or whether the milk secretion declines in accordance with the ability of the mother. The fact that the decline sets in before the young open their eyes certainly indicates that the activity of the young is not the only factor involved.

This much, however, seems clearly indicated: the break in the growth curve of the suckling mouse is not due to a succession of cyclic processes inherent in the young mouse (compare Robertson, '28). In sharp contrast to the view employed by Robertson is the idea (Crozier, '26) that the growth curve may reflect the operation of an essentially unitary process throughout the progress of development, and the appearance of superimposed cycles of increase may be due to temporary, self-imposed deficiencies in growth rate. In the case of the rat and other forms (Crozier, '26; and in MSS.), it was suggested that deviations of the observations from the calculated curve during such a depression in growth rate may be dealt with as a subsidiary curve, perhaps expressing the result of activity of thyroid. The present work on the mouse shows that the depression in growth rate at the end of the second week after birth is due to external limitation imposed upon the course of development, and that this limitation is chiefly connected with the amount and nature of the food received.

I wish to express my gratitude for the support and advice given to this study by Prof. W. J. Crozier and Dr. G. Pineus, of this laboratory.

SUMMARY

1. The daily amount of milk secreted by lactating mice was estimated by weighing the young before and after feeding. The daily losses in feces, urine, and from other sources were determined similarly.

2. The milk curve constructed upon the observations rises from parturition until about the tenth day and declines from then until the completion of weaning. The daily losses show a steady increase with the age of the young.

3. When the litter size is varied, the amount of milk produced by lactating females is not proportional to the number of young in a litter, but to some power of it.

4. The gain in weight of the suckling young depends chiefly on the amount of milk they receive; the shape of the milk curve therefore explains to a large extent the depression in the growth curve occurring near the end of the second week after birth.

LITERATURE CITED

- BRODY, S., A. C. RAGSDALE, AND C. W. TURNER 1923 *J. Gen. Physiol.*, vol. 5, p. 777.
- BRODY, S., C. W. TURNER, AND A. C. RAGSDALE 1924 *J. Gen. Physiol.*, vol. 6, p. 541.
- BROWN, L. A. 1926 *J. Gen. Physiol.*, vol. 10, p. 111.
- 1927 *J. Gen. Physiol.*, vol. 11, p. 37.
- CROZIER, W. J. 1926 *J. Gen. Physiol.*, vol. 10, p. 53.
- DAVENPORT, C. B., AND W. W. SWINGLE 1927 *J. Exp. Zool.*, vol. 48, p. 395.
- ENZMANN, E. V., AND G. PINCUS 1933 *Am. J. Physiol.*, vol. 103, p. 30.
- ENZMANN, E. V., N. R. SAPHIR, AND G. PINCUS 1932 *Anat. Rec.*, vol. 54, p. 325.
- ENZMANN, E. V., AND W. J. CROZIER In press.
- HOLT, L. E., AND J. HOWLAND 1922 *Diseases of infancy and childhood*. New York and London.
- GAINES, W. L., AND F. A. DAVIDSON 1926 *J. Gen. Physiol.*, vol. 9, p. 325.
- GATES, W. H. 1925 *Anat. Rec.*, vol. 29, p. 183.
- GOWEN, J. W. 1920 *Maine Agric. Exp. Station Bull.*, vol. 293, p. 185.
- JUDSON, S. A. 1916 *Yale Univ. Dissert.*
- MACDOWELL, E. C., W. H. GATES, AND C. G. MACDOWELL 1930 *J. Gen. Physiol.*, vol. 13, p. 529.
- OSTWALD, W. 1908 *Vortr. und Aufs. über Entwmech.*, Bd. 5.
- ROBB, R. C. 1929 *Brit. J. Exp. Biol.*, vol. 6, p. 293.
- ROBERTSON, T. B. 1916 *J. Biol. Chem.*, vol. 24, p. 363.
- 1928 *J. Gen. Physiol.*, vol. 8, p. 463.
- SALLER, K. 1927 *Arch. Entwmech.*, Bd. 111, S. 453.
- TURNER, C. W. 1931 *Mo. Agric. Exp. Res. Bull.*, vol. 156.
- 1932 *The mammary glands*. Reprinted from "Sex and internal secretions," p. 544.
- WEATHERFORD, H. L. 1929 *Am. J. Anat.*, vol. 44, p. 199.

THE CEREBROSPINAL FLUID AND THE CERVICAL LYMPH NODES

O. A. MORTENSEN AND W. E. SULLIVAN
Department of Anatomy, University of Wisconsin

ONE PLATE (TWO FIGURES)

That the cerebrospinal fluid passes both into the venous sinuses and into the cervical lymph nodes has been adequately demonstrated by Key and Retzius and more recently by Weed and his co-workers. A review of the literature is unnecessary, as the latter group has covered it in detail. We simply wish to point out that there are now available two agents with which one can demonstrate readily in the living that foreign material introduced into the subarachnoid space appears after a short period of time in the cervical lymph nodes both superficial and deep.

The early work was with a brominized oil (brominol, light), the later with thorium dioxide (thorotrast) and it will be most convenient to describe the work in terms of these two agents. Dog no. 3 will illustrate what may occur with brominol; dog no. 12 with thorotrast.

DOG NO. 3. BROMINOL

Using nembutal for anaesthesia, a cisternal puncture was made in a female of about 14 pounds. Slightly less than 3 cc. of clear cerebrospinal fluid was withdrawn and replaced by the brominized oil. A roentgenogram taken 30 minutes later showed the oil along the cervical cord and along the base of the brain. There is some diffusion of the oil in the posterior cranial fossa. At the end of the second hour there is a little more spreading over the cerebral hemispheres and the optic nerves are outlined. At the end of the third and fourth hours

the cerebral region shows no appreciable change, but the nasal region is absorbing more x-ray. At the end of the fifth hour enough of the brominized oil had reached the upper deep cervical nodes so that they were differentiated in the roentgenogram from the neighboring tissues. These nodes lie just caudal to the tympanic bulla and ventral to the atlas. At the end of the sixth hour these nodes are more clearly outlined and the submaxillary nodes together with a group opposite the third cervical vertebra can be recognized. The infiltration of the nodes continues at least to the end of the seventy-fifth hour, the increase in the submaxillary group being rather greater than in the deep cervical.

This represents in a general way our experience with the oil. In no dog were the nodes demonstrable in less than 5 hours, and in two instances it could not be demonstrated in the nodes even after several days. In interpreting the time element, however, it must be kept in mind that the oil is heavy, inert, and non-miscible with the tissue fluids and that it must be present in the nodes in quantity before it is demonstrable by x-ray.

It is interesting that there could be a filling of either the deep or superficial nodes in a particular animal and also that the filling was not necessarily bilateral.

DOG NO. 12. THOROTRAST

A cisternal puncture was made as before and 30 drops of clear fluid were permitted to escape from the needle. This was replaced by slightly less than 2 cc. of thorotrast. A roentgenogram made 5 minutes later showed a rather uniform dispersal of the thorotrast around the brain and cervical cord. This is in marked contrast to the brominol, which tends to be limited to the base of the brain. At the end of 30 minutes, the convolutions of the brain are clearly outlined, the optic nerve is seen and the upper deep cervical nodes are differentiated from the neighboring tissues. The nodes are rather more distinct than in the brominol dog at 5 hours. It is also obvious that the nasal region is absorbing more

x-ray. At 90 minutes the cervical nodes are infiltrated as far caudad as the third vertebra and the submaxillary group can be recognized. In a roentgenogram at the end of the sixth hour (fig. 2), it is easy to recognize vessels entering the cephalic end of the nodes. Somewhere in this period the picture of the nodes become relatively stable and remains so for at least 2 months. There are detailed but not significant changes.

As an aid in the interpretation of the roentgenograms, gelatin injections of the subarachnoid space were made. On dissection it became clear that the deeper nodes were filling by way of the nasal cavity. This route has been demonstrated by earlier workers using the gelatin method, and it is only necessary to review the fact that the mass passes from the subarachnoid space along the olfactory nerves to the nasal mucosa and then by way of lymph vessels along the pharynx to the upper deep cervical nodes. The submaxillary nodes are filled by vessels running subcutaneously. We were able to trace these vessels only as far as the bucconasal junction and were inclined to interpret them as coming from the terminal part of the nose. Wustmann, however, has demonstrated that vessels coming from the orbit pass along the angular vein and then take much the course we have just described. The fact that in some animals there was a filling of the submaxillary nodes without a filling of the deep nodes would support his interpretation.

It does not seem necessary to describe the effects of the brominol on the animals beyond saying that they showed symptoms similar to those described by Davis, Haven, and Stone in their use of iodized oil.

Our experience with thorotrast differed from Wustmann's. His animals died when he used the standard formula. Our animals showed rather less reaction than with the brominol. Comparing methods, it may be pointed out that he used 8 cc. of thorotrast and does not state whether fluid was withdrawn. We used only 2 to 3 cc. to replace an equal amount of fluid. The latter amount seems entirely adequate. With a modi-

fied preparation he got excellent results and while he does not record a filling of the nodes until the end of the thirty-fourth hour, it is obvious from his illustration that he could have demonstrated it earlier.

SUMMARY

Two agents, now available, make it possible to demonstrate in the living animal that foreign material introduced into the subarachnoid space passes rather readily into the cervical lymph nodes both superficial and deep. Thorium dioxide is transferred more rapidly than brominized oil and is more satisfactory from the standpoint of roentgenology.

LITERATURE CITED

- KEY AND RETZIUS 1876 *Anatomie des Nervensystems und des Bindegewebes*. Stockholm.
- WEED, LEWIS H. 1922 *The cerebrospinal fluid*. *Physiological Reviews*, vol. 2, no. 2, p. 171, April.
- DAVIS, HAVEN, AND STONE 1930 *The effect of injections of iodized oil in the spinal subarachnoid space*. *J. A. M. A.*, vol. 94, p. 772. March.
- WUSTMANN, O. 1933 *Experimentelle Untersuchungen über die Reliefdarstellung (Umrisszeichnung) des Zentralnervensystems im Röntgenbild durch Thoriumkontrastmittel*. *Deutsche Zeitschrift für Chirurgie*, 238 Bd., 9. und 10. Heft, January.

PLATE 1

EXPLANATION OF FIGURES

- 1 Head and neck of normal dog (no. 12).
- 2 Same dog, 6 hours after injection of thorotrast.



THE MECHANISM OF INTAKE AND OUTFLOW OF FLUIDS IN THE LATERAL LINE CANAL OF FISHES¹

GEORGE MILTON SMITH

Anatomical Laboratory, School of Medicine, Yale University

ONE PLATE (THREE FIGURES)

In an earlier paper (Smith, '30)² it was shown that there existed a mechanism of intake of fluids into lateral line canals of the goldfish, with subsequent outflow. This could be readily demonstrated (fig. 1) by the use of colored fluids, such as suspension in India ink, vermilion, and Berlin blue, either by applying drops of these colored fluids by means of a pipette to the head region of the fish or by permitting the fish to live in a colored suspension for varying lengths of time (fig. 2).

The intake of colored suspension into the canal system, as seen with the aid of a dissecting microscope, was found to be a rapid process, varying from a few seconds to a few minutes. The outflow from the canals was slower, taking usually from 15 minutes to an hour or more before complete clearing of the canals occurred.

The present paper, suggested by Prof. A. E. Parr, seeks to extend the observation on the filling and emptying process of the lateral line canals to fishes other than the goldfish. The study was carried out on a variety of living fishes available in the laboratories connected with the Yale Department of Anatomy, the New York Aquarium, and the Bermuda Marine Biological Station. It is a pleasure at this time to acknowledge the kind cooperation of Dr. Charles M. Breder and

¹ Aided by grant from Blossom Fund, Yale University. Experiments conducted at the Bermuda Biological Station and the New York Aquarium through the courtesy of these institutions.

² Smith, G. M., 1930, A mechanism of intake and expulsion of colored fluids by the lateral line canals as seen experimentally in the goldfish (*Carassius auratus*). *Biological Bull.*, vol. 58, p. 313.

Dr. C. W. Coates, of the New York Aquarium, and Dr. John G. Wheeler, of the Bermuda Marine Biological Station, in permitting the use of their collections for these experimental purposes.

The method employed on sixty-five different kinds of fishes was essentially that used in the earlier experiments, except that several drops of water color Ivory Black³ solution was used.

It became evident in the course of the experiments that a large number of fishes failed to show definitely this mechanism of intake and outflow of colored suspensions. The failure of colored fluids to visibly penetrate into the lateral canal system can be explained partly to difficulties caused by the deep anatomic position of the canals lying in the bony structure of the head or to heavy black pigmentation of skin overlying the canals, both causes rendering observations difficult or impossible. At times, an abundance of cutaneous mucus acted to prevent the penetration into lateral canals of the solutions used in the experiment. On the other hand, it is not unlikely that, for chemical or physical reasons as yet not appreciated, a penetration of the colored fluids employed did not occur.

Thus, in the following fishes no definite penetration of colored fluids into lateral line canals was noted, the experiments being carried out only on single or very few specimens in each instance:

Tautoga onitis, *Lutianus apodus*, *Monacanthus hispidus*, *Chilomycterus schoepfii*, *Tautogolabrus adspersus*, *Haemulon sciurus*, *Haemulon plumeri*, *Haemulon flavolineatum*, *Anisotremus virginicus*, *Bathystoma striatum*, *Lutianus synagris*, *Spheroides maculatus*, *Hippocampus hudsonius*, *Cynoscion regalis*, *Opsanus tau*, *Prionotus strigatus*, *Leiostomus xanthurus*, *Aleutera schoepfii*, *Melichthys bispinosus*, *Iridio bivitatus*, *Pogonias cromis*, *Salvelinus fontinalis*, *Ameiurus nebulosus*, *Morone americana*, *Pomoxis annularis*, *Oncorhynchus tshawytscha*, *Dormitator maculatus*, *Dormitator latifrons*, *Trichogaster trichopterus*, *Macropodus opercularis*,

³ Water color Ivory Black (Winsor and Newton, Limited, London, England) 1 tube contents, weight approximately 7 gm., dissolved in 2000 cc. fresh or sea water.

Ambassis lala, Rivulus, Doras chataphractus, Corydoras palleatus, Channa fasciata.

In contrast with the above-mentioned group, a considerable number of different fishes, enumerated in the table below, showed clearly a filling of lateral line canals when pigmented solutions were applied to the nasal region. The intake of colored fluids was rapid, resembling that of the goldfish already described. The disappearance of colored suspension from the canals was invariably much slower and varied over a wide range.

In the following table, the outflow of colored fluids from the canal system of single specimens has been tabulated.

NAME OF FISH	SIZE IN INCHES	TIME OF OUTFLOW	LABORATORY
		<i>Minutes</i>	
Poronatus triacanthus	5	20	New York Aquarium
Diplectrum formosum	4	60	New York Aquarium
Perca flavescens	5	3	New York Aquarium
Lepomis auritus	5	30	New York Aquarium
Lepomis pallidus	3	10	New York Aquarium
Eupomotis gibbosus	3	5	New York Aquarium
Catostomus commersonii	6	1	New York Aquarium
Ambloplites rupestris	7	15	New York Aquarium
Tinca vulgaris	5	12	New York Aquarium
Myeteroperca falcata	10	2	New York Aquarium
Epinephelus adscensionis	9	10	New York Aquarium
Mugil curema	2	2	New York Aquarium
Abramis crysoleucas	7	11½	New York Aquarium
Scardineus erythrophthalmus	6	15	New York Aquarium
Stenotomus chrysops	1½	11.5	New York Aquarium
Centropristis striatus	8	10	New York Aquarium
Fundulus heteroclitus	2	1	New York Aquarium
Carrasius auratus	7	12	New York Aquarium
Aequidens latifrons	1½	2	New York Aquarium
Astyanax bimaculatus	2½	10	New York Aquarium
Astyanax fasciatus	1½	15	New York Aquarium
Barbus conclonicis	1¾	40	New York Aquarium
Hemigrammus	1½	10	New York Aquarium
Danio malabaricus	1½	3½	New York Aquarium
Rasbora meinkei	1½	15	New York Aquarium
Characinus uncinatus	1	19	New York Aquarium
Panchax chareri	1	3	New York Aquarium
Eucinostomus havana	1½	200	Bermuda Biological Station
Holocentris ascensionis	2	70	Bermuda Biological Station
Angeliethys	7	200	Bermuda Biological Station

It was found that some of the smaller so-called tropical fishes could be used for a study of the filling of the lateral line canals. Figure 3 shows a specimen of *Hemigrammus* with head and trunk canals filled with India ink by applying this substance to the terminal canals in the nasal region.

A discussion with bibliography of this subject appeared in the previous publication to which reference has been made.

SUMMARY

The inflow of fluids, artificially colored, into the lateral line canals and the subsequent outflow is described in a variety of fishes. These observations are similar in character to those found to exist in the goldfish, already reported, and suggest that the lateral line canals furnish a device for testing physical or chemical changes of aquatic environment.

PLATE

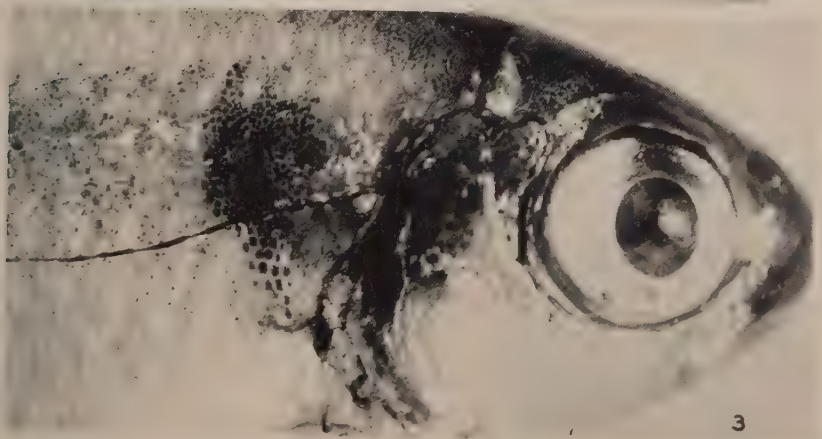
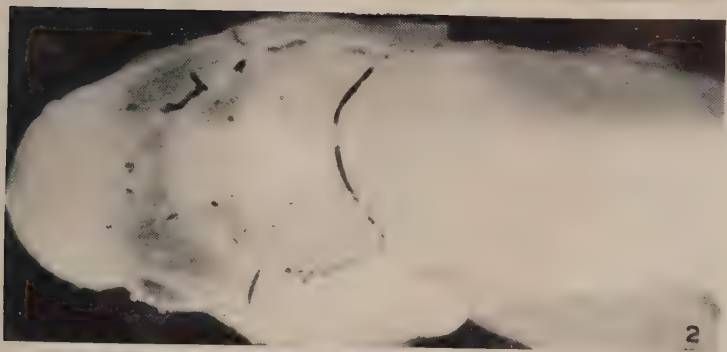
PLATE 1

EXPLANATION OF FIGURES

1 *Carassius auratus*, with lateral line canals of head filled by placing a few drops of India ink in contact with terminals of lateral line canals at the olfactory depression. Photograph of living fish. $\times 4$.

2 *Carassius auratus*, with lateral line canals irregularly filled with vermilion suspension, in which fish lived for 2 months. Photograph of specimen fixed in formalin. $\times 4$.

3 *Hemigrammus*, lateral line canals filled by applying a few drops of India ink to terminals of supra- and infra-orbital canals near the olfactory depression. Photograph of living fish. $\times 12$.



CELLULAR CONSTITUENTS OF THE ANTERIOR HYPOPHYSIS AFTER UTERINE IMPLANTS OF CARCINOMA IN RATS

M. F. GUYER AND P. E. CLAUS

Department of Zoölogy, University of Wisconsin

TWO TEXT FIGURES AND ONE PLATE (SEVEN FIGURES)

In an earlier study ('32) we found that, following subcutaneous implants of 'Flexner-Jobling carcinoma,' a marked increase in the number of basophile cells in the anterior lobe of the hypophysis occurred, with no perceptible change in the oxyphiles. Much the same histological picture resulted as that which follows castration, characterized not only by increased number of basophile cells, but by some degree of vacuolization. Increased ovarian size and general sexual precocity, furthermore, beyond that induced by transplants of normal anterior pituitary substance, were inaugurated in immature mice to which such hypophysial material was transplanted.

The present study has to do with the effects of uterine implants of this same carcinoma. While we found it more difficult to get such grafts to grow in the uterus than subcutaneously—approximately 50 per cent as compared with 80 to 90 per cent—this is probably due in part to the much greater technical difficulties involved. Once the tumorous transplants become established in the uterus, however, they grow much more rapidly and to larger size than when implanted beneath the skin. The fuller blood supply to be found in and near the uterine tissue is doubtless an important factor in this riotous growth which is also accompanied by profuse metastasis.

Sections of the pituitary bodies of the eight individuals having such uterine cancers display a different histological picture from that shown by rats with subcutaneous tumors. There is considerably less development of extra basophile cells and a noticeable increase in the number of oxyphile cells (fig. 2). For purposes of comparison, sections of the hypophysis from all individuals were made as nearly as possible in the same plane and were cut either of 7 or of 3 μ thickness. In this way corresponding regions in the several anterior lobes could be compared as to numbers and types of cells. Estimates based on 100 counts indicate that the increase in oxyphile cells in the anterior pituitary glands of rats with well-established uterine tumor is from 35 to 50 per cent, when compared with similar areas in normal rats.

Such an increase in oxyphile cells, of course, suggests the condition that is common in pregnant rats and is markedly displayed in pregnant mice. The question arises whether these additional oxyphiles are true 'pregnancy cells'—a type alleged by some observers to be distinct from the ordinary oxyphile cells—or merely additional oxyphiles of the usual type. We have been led by our observations to the latter conclusion, but also after a parallel study of normal pregnant controls we are doubtful of the distinctions which are alleged to exist between the ordinary oxyphile cells and the so-called pregnancy cells in the rat. This is a point to which we have devoted considerable attention, using a Severinghaus technique ('32), the familiar Mallory's triple connective tissue stain, and various methods for demonstrations of the Golgi apparatus. While one can on occasion see here and there the two types of oxyphiles stressed by Haterius ('32), there are so many intergradations that we were unable to satisfy ourselves that we were dealing with two distinct types, but felt rather that we were merely viewing variants of the same kind of cell. While our attempts to use the easily demonstrated Golgi apparatus as a basis of classification were fairly successful in discriminating between oxyphiles and basophiles (figs. 6 and 7), as described by Severinghaus ('32) for the rat,

and by Atwell ('32) for the cat, they yielded no convincing results in identifying two distinct types of oxyphiles.

Several females were castrated and at the same time implanted with uterine cancer. These castrates showed the same increase in basophile cells in the anterior hypophysis as do ordinary castrates, but some fifty counts showed that while there was some increase in oxyphiles (fig. 5), it was not so great as in uncastrated rats with uterine cancer (fig. 2). The number of oxyphiles was but slightly greater than that found in normal non-pregnant rats.

Although various investigators (Zondek and Berblinger, '31; Hohlweg and Dorn, '31; and Nelson, '33) have reported that the increase of basophiles in the anterior hypophysis is prevented in the castrate animal by successive injections of oestrin, such inhibition of basophile formation did not occur in our animals. The discrepancy between our results and those of the investigators referred to is due doubtless to differing techniques in the extraction of the oestrin. We used theelin, the Parke Davis & Co. preparation of oestrin, a water-soluble product. Nelson informs us that he used an oil-soluble extract which probably is less rapidly oxidized in the body than is the water-soluble form. Some of our castrated female rats were for 6 weeks given 4 rat units daily of theelin, 2 units in the morning, 2 in the evening. That our preparation was potent is shown by the fact that such dosage kept the rats in a condition of almost continuous oestrus as indicated by vaginal smears; nevertheless, they developed a marked increase in the number of basophile cells in the anterior lobe of the pituitary body. Such daily injections of theelin, moreover, were begun in these animals before castration. A glance at fig. 3, which is a photograph of a section from the anterior lobe of an oestrin-treated castrate, shows such an excess number of basophiles.

Likewise in non-castrated individuals with subcutaneous cancers, daily injections of 4 rat units of theelin kept the females in an almost constant state of oestrus (text fig. A) and did not prevent the development of extra basophile cells,

although about the same increase in the number of oxyphile cells (fig. 4) was induced as in the cases of non-injected rats with uterine cancer. It is evident, therefore, that increase in oxyphile cells follows injections of oestrin, uterine carcinoma, or pregnancy.

While in the cases of rats with uterine implants of cancer the increase in oxyphile cells in the anterior lobe of the hy-

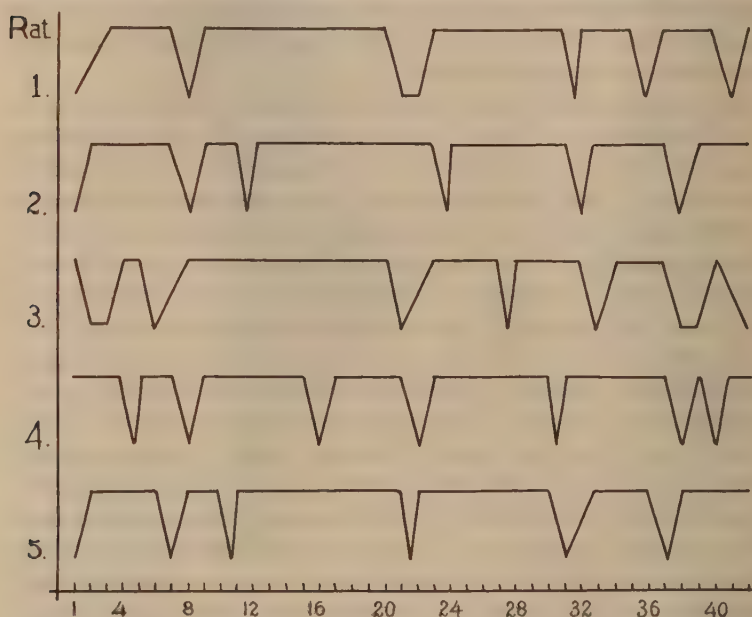


Fig. A Graphs showing the oestral cycles in five rats, with subcutaneous cancer, injected daily with oestrin (theelin); the upper level indicates oestrus, the lower level, dioestrus. The rats remained in almost constant oestrus.

pophysis simulates the condition typical of pregnancy, the regular sexual rhythm seemed to be but little disturbed (text fig. B). Immediately following implantation there might, in some cases, ensue a short period of dioestrus, due possibly to traumatic injury, but usually, as shown by daily smears, the regular oestral cycle was promptly resumed. Sections of the ovaries of such cancerous individuals, moreover, commonly

showed the usual picture of the normal ovary of the mature, non-pregnant rat, with young growing follicles and corpus luteum residues.

SUMMARY

1. Following implants of the Flexner-Jobling carcinoma in the uterus of rats, there is a slight increase in the number of basophile and a marked increase in the number of oxyphile cells in the anterior lobe of the pituitary body. The histological picture is the same as that which is associated with pregnancy or with injections of oestrin.

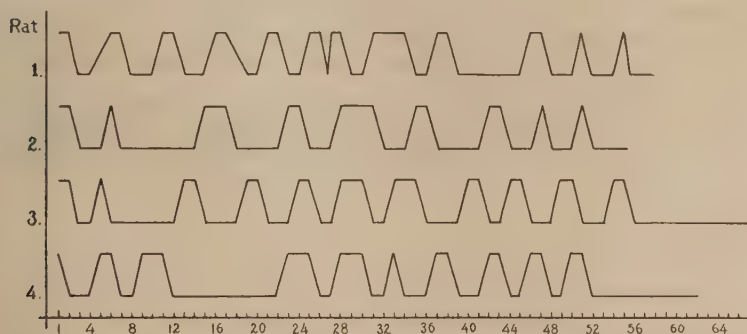


Fig. B Graphs showing the oestral cycles in four rats with uterine cancer; the upper level indicates oestrus, the lower level, dioestrus. The oestral rhythm was interfered with but little.

2. Following such implants in castrated females, there is not so great an increase in oxyphile cells.

3. Daily injections of 4 rat units of a water-soluble oestrin (theelin) does not prevent the formation of castration cells in the hypophysis of either castrated animal or those with subcutaneous cancer, although such treatment keeps the females in a state of almost continuous oestrus.

4. The oestral cycle continues in rats with transplanted uterine carcinoma and sections of the ovaries of such individuals disclose the usual histological picture of the non-pregnant rat.

LITERATURE CITED

- ATWELL, WAYNE J. 1932 Characteristics of the Golgi apparatus in the different types of cells of the anterior hypophysis. *Anat. Rec.*, vol. 55, no. 1, p. 11.
- GUYER, M. F., AND P. E. CLAUS 1932 Cell changes in the anterior lobe of the pituitary gland following cancer transplantation in rats. *Anat. Rec.*, vol. 52, no. 3, p. 225.
- HATERIUS, H. O. 1932 The relation of pregnancy cells in the pituitary of the rat to the reproductive cycle. *Anat. Rec.*, vol. 54, no. 3, p. 343.
- HOHLWEG, W., AND M. DOHRN 1931 Beziehung zwischen Hypophysenvorderlappenhormone und Keimdrüsen. *Wien. Arch. f. innere Med.*, Bd. 21, S. 337.
- NELSON, WARREN O. 1933 The prevention of castration changes in the rat hypophysis by the administration of oestrin and of testis hormone. *Anat. Rec.*, vol. 55, no. 4 (sup.), p. 31.
- SEVERINGHAUS, AURA E. 1932 a A cytological technique for the study of the anterior lobe of the hypophysis. *Anat. Rec.*, vol. 53, no. 1, p. 1.
- 1932 b Cytological studies on the anterior pituitary. *Anat. Rec.*, vol. 53, no. 2 (sup.), p. 35.
- ZONDEK, B., AND W. BERBLINGER 1931 Der Einfluss des weiblichen Sexualhormons und der Hypophysenvorderlappenhormone auf die Struktur der Ratten- und Mäuse-hypophyse. *Klin. Wehnschr.*, Bd. 10, S. 1061.

PLATE

PLATE 1

EXPLANATION OF FIGURES

1 Photograph of section showing the general appearance of cells—a few basophiles (the larger cells), some oxyphiles, but mainly neutrophiles—in the anterior lobe of the hypophysis in the normal rat.

2 Photograph showing increase in number of oxyphile cells (deeply stained) in the anterior lobe of an uncastrated rat with uterine cancer.

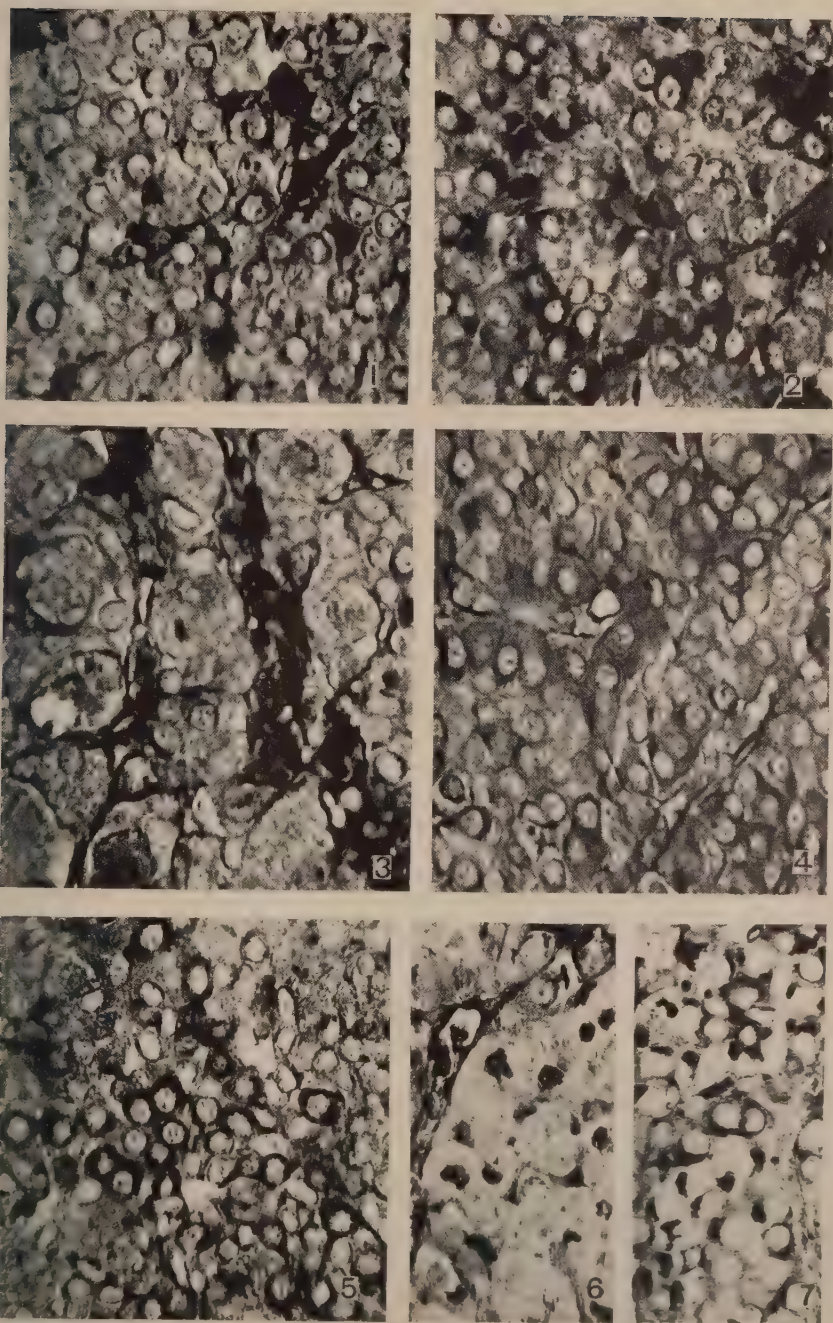
3 Photograph showing castration cells in anterior hypophysis which have not been inhibited by daily administration of oestrin (theelin).

4 Photograph showing increase in hypophysial oxyphile cells (more deeply shaded) after injections of oestrin into rats with subcutaneous cancer.

5 Photograph of anterior hypophysial tissue from a castrate rat with uterine cancer which showed a certain amount of increase in the number of both basophile and oxyphile cells.

6 Photograph showing Golgi apparatus (in black) mainly in basophile cells.

7 Photograph showing Golgi apparatus (in black) of smaller type than that seen in 6, mainly in oxyphile cells.



THE FISSULA ANTE FENESTRAM IN AN ADULT HUMAN EAR¹

BARRY J. ANSON AND J. GORDON WILSON

*Departments of Anatomy and Otolaryngology, Northwestern University
Medical School*

FOUR PLATES (THIRTEEN FIGURES)

It has long been known that there exists within the lateral wall of the osseous vestibule a minute channel which extends from the tympanic cavity inward through the bone to a point just in front of the vestibular window. Its course is seen in Siebenmann's² (1890) corrosion preparations. Perozzi ('16) described the appearance of the channel as observed in serial sections from fetuses, infants, and adults; he drew attention to its connective-tissue content, which he and certain later investigators regarded as a persisting synchondrosis between the primordial cochlear and vestibular parts of the cartilaginous otic capsule. Bast recently figured the structure as it appeared in sections of the ear from fetuses and from two adults (of 40 and of 70 years); he has also modeled the fissular space in six fetal ears ('30; footnote 2). Our study is limited to the form and content of the fissula in the adult human ear, as shown by models particularly.

¹ Contribution no. 186 from the Anatomical Laboratory of Northwestern University Medical School. An investigation conducted under the auspices of the American Otological Society. Reported at the Cincinnati meetings of the American Association of Anatomists; abstract, *Anat. Rec.*, vol. 55, no. 4, p. 4, March, 1933.

² This and other accounts have been discussed by Doctor Bast in a recent work, and need not be reviewed here (T. H. Bast, Ossification of the otic capsule in human fetuses. *Contr. to Embr.*, no. 121, Extr. from *Publ. no. 407*, *Carn. Inst.*, Wash., June, 1930).

MATERIAL

The specimen herein described was removed from the left temporal bone of an adult, 18 years of age. The block was fixed and stored in 5 per cent formalin and decalcified in a dilute solution of picric acid in 10 per cent formalin. Celloidin sections were cut at thicknesses varying between 20 and 30 μ ; they were stained in either hematoxylin and eosin or in van Gieson's stain. The plane of section was approximately horizontal. Models from the series were made by the Born wax-plate method from tracings prepared with an Edinger projection-apparatus at a magnification of 50 and of 200 diameters. The models, showing both the fissular area of the bone and the cavity of the fissula itself, consist of sixty-six plates representing sections between nos. 67 and 132, inclusive.

OBSERVATIONS

Considered as a channel within the bone, the fissula ante fenestram began, in our specimen, as a crevice-like continuation of the vertical sulcus, which on the labyrinthic or medial wall of the tympanic cavity, marks the posterior extent of the promontory (fig. 4; compare figs. 6 and 7). This tympanic orifice of the fissula is situated above and in front of the vestibular window; it measures 0.06 mm. in width and 0.265 mm. in vertical length.³ After traversing the bone in an inferior and posterior (dorsal) direction, the opposite extremity becomes continuous with the cavity of the internal ear just medial to the anterior extremity of the vestibular window, at a point where the vestibule becomes the scala vestibuli (fig. 5; compare with figs. 6 and 10); here the cleft is 0.06 mm. wide superiorly, 0.2 mm. in its midportion, and below blends with the scala; it is 0.34 mm. long (the latter, estimated). As may be seen in the sectional model (figs. 7 to 10), the long axis of the fissula changes through approximately 90° between its two extremities, being at first directed inward (fig. 7), but, while still in the superior fourth of its course, turning backward (fig. 8)—which plane is maintained to the termination (fig. 10).

³ Length estimated from number of sections through which the structure passed.

The form of the fissula is more effectively displayed by the model (figs. 11 to 13) which represents a cast of the cleft-like space enclosed by the walls of the aperture. Both its anterior and the posterior margins are concave backward, and its outline therefore falciform. The space is not wholly independent of the haversian canal-system of the neighboring bone, since from the anterior margin at several points (figs. 11 to 13) communications are established between the two; similar connections occur on the medial (or vestibular) aspect, an example of which may be seen in the section photomicrographed as figure 1, and at an inferior level in the model photographed for figure 8. Within the connective tissue which fills these spaces, small vessels—one to each communication, and a total of about nine—enter or leave the fissula. No fissular vessels in our series were of direct periosteal origin.

The tissue forming the walls of the fissula at its superior part (fig. 1) is largely bone; midway in the course (fig. 2), the fissula rests in modified cartilage (interglobular spaces or intrachondral bone); near the inferior limit (fig. 3), cartilage is again largely replaced by bone. Here, where the fissula joins the vestibule, the fibers of the stapedial ligament enter it and blend with the contained connective tissue.

A further study of the fissula is in progress; the structure is being modeled as it appears in serial sections of the ear from infants, children, and older adults.

ABBREVIATIONS

<i>bas.st.</i> , basis stapedis (base or foot-plate of stapes)	<i>f.a.f.</i> or <i>fiss.a.f.</i> , fissula ante fenestram
<i>cav.t.</i> , cavum tympani (tympanic cavity or middle ear)	<i>lig.st.</i> , lig. annulare baseos stapedis (annular ligament of stapes)
<i>cochl.</i> , cochlea	<i>meat.a.i.</i> , meatus acusticus internus (internal acoustic meatus)
<i>cu.</i> , cupula (cupula or apex)	<i>vest.</i> , vestibulum (vestibule)
<i>fen.v.</i> , fenestra vestibuli (vestibular or oval window)	

PLATE 1

EXPLANATION OF FIGURES

Photomicrographs of horizontal sections through the ear of an 18-year-old male. Magnification, 74 diameters.

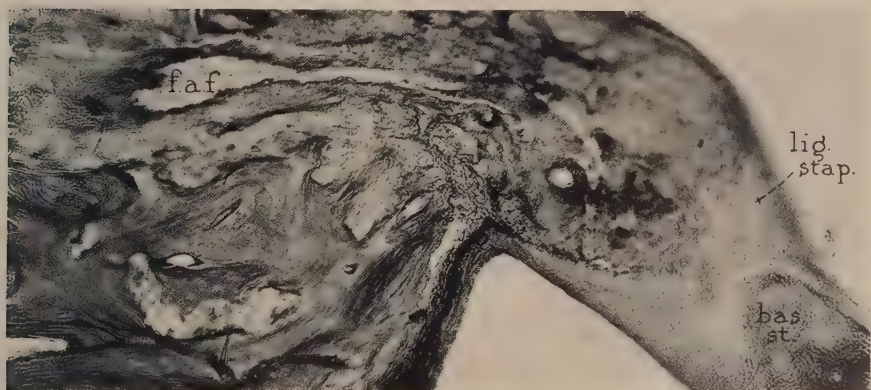
1 Section (no. 75 in the series) showing the communication between the fissula ante fenestram and the tympanic cavity (middle ear), and connection with a haversian canal. The section is at a level inferior to that represented by the top of the model in figure 7.

2 Section (no. 100) showing the form of the fissula in the lower third of its course. This level is represented by the displayed surface of the model photographed in figure 9. The fissula is surrounded by intrachondral bone (so-called interglobular spaces).

3 Section (no. 112) showing the communication between the fissula ante fenestram and the vestibule of the internal ear, near the margin of the oval window. This level is represented by the upper surface of the model photographed in figure 10. The stapedial ligament is continuous with fibrous tissue in the fissula.



1



2



3

PLATE 2

EXPLANATION OF FIGURES

Photographs (two views) of a model of part of the osseous labyrinth, showing the relation of the fissula ante fenestram to the adjacent portions of the tympanic cavity, vestibular window, vestibule, cochlea, and internal acoustic meatus. The original model represents a magnification of 50 diameters; the photographs, of 16 diameters.

4 Superior and lateral view of the model at a level represented by the top of the sectional model (fig. 7), showing the position of the tympanic (or superior) extremity of the fissula, above and in front of the oval window.

5 Inferior and lateral view of the model, at level represented by the bottom of the sectional model (fig. 10), showing the location of the vestibular extremity of the fissula, below and in front of the oval window.

The large model in the succeeding plate (fig. 6) includes, in more detailed manner, the immediate area of the fissula ante fenestram.

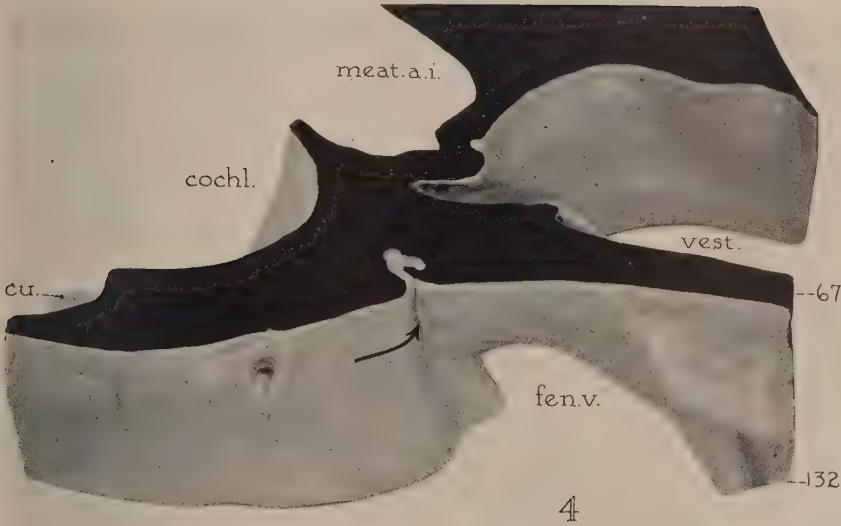


PLATE 3

EXPLANATION OF FIGURES

6 Photograph of a model of the bony labyrinth in the region of the fissula, viewed from a posterior (vestibular) and somewhat superior position (in relation to the location of the area in the temporal bone). On the lateral (tympanic) aspect, and the top of the model (compare fig. 7), is seen one extremity of the fissula; on the medial (vestibular) aspect, below, the other extremity is seen (compare fig. 10), situated just in front of the anterior limit of the oval window. The curved arrows point to the orifices at the two extremities of the fissure; the straight arrow passes from the tympanic cavity to the vestibule through the anterior part of the vestibular window (stapes omitted in model). The original model represents a magnification of 200 diameters; the photograph, of 48 diameters.

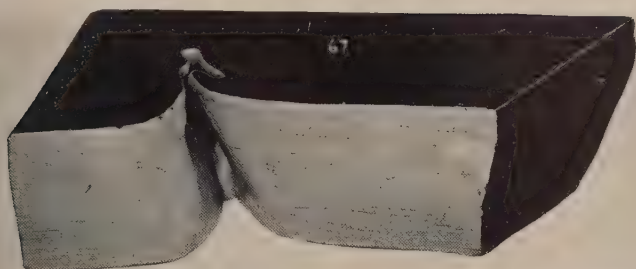
7 to 10 Photographs of the superior aspect of each of four levels of the sectioned model seen in figure 6, showing the region of the fissula ante fenestram. The original model represents a magnification of 200 diameters; the photographs, of 44 diameters.

7 Uppermost block of model, viewed from a lateral and somewhat superior position (in relation to the location of the structures in the temporal bone). The upper surface represents section no. 67 in the series. The tympanic end of the fissula ante fenestram is shown, as it opens into the depths of an elongate vertical recess.

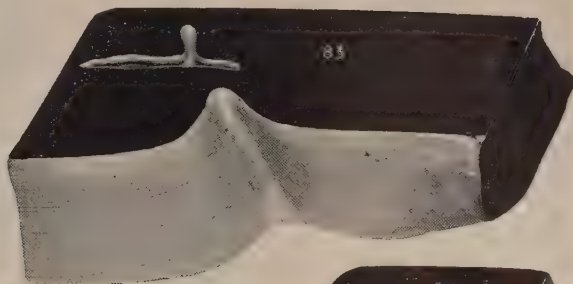
8 The succeeding (or second) block of the model, similarly viewed. The superior surface represents section no. 83. The long axis of the fissula has changed; its characteristic slit-like form is evident. Its space communicates medially with a haversian canal.

9 The third block of the sectioned model; the upper surface represents section no. 100, seen in the photomicrograph, figure 2. The fissula is approaching its inferior or vestibular end.

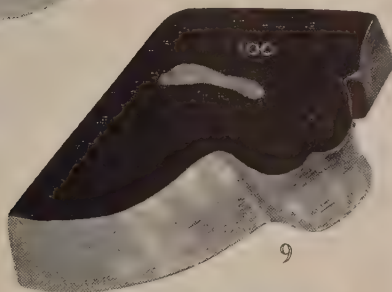
10 The lowermost (fourth) block of the model, viewed as in the preceding figures; the superior surface is section no. 112, photomicrographed in figure 3. The inferior or vestibular extremity of the fissula is seen as it communicates with the internal ear near the anterior margin of the oval window.



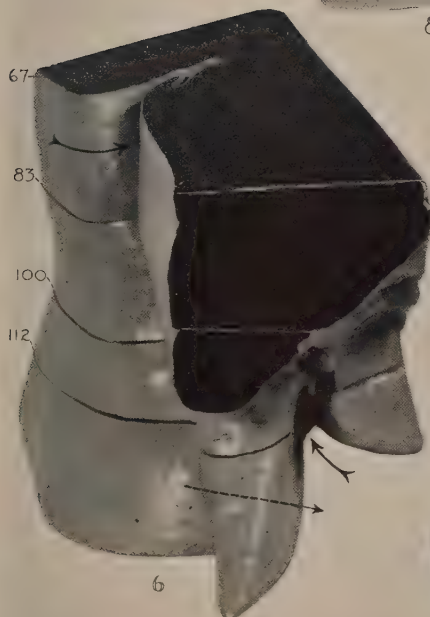
7



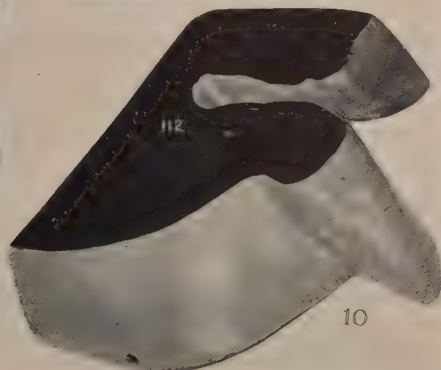
8



9



6



10

PLATE 4

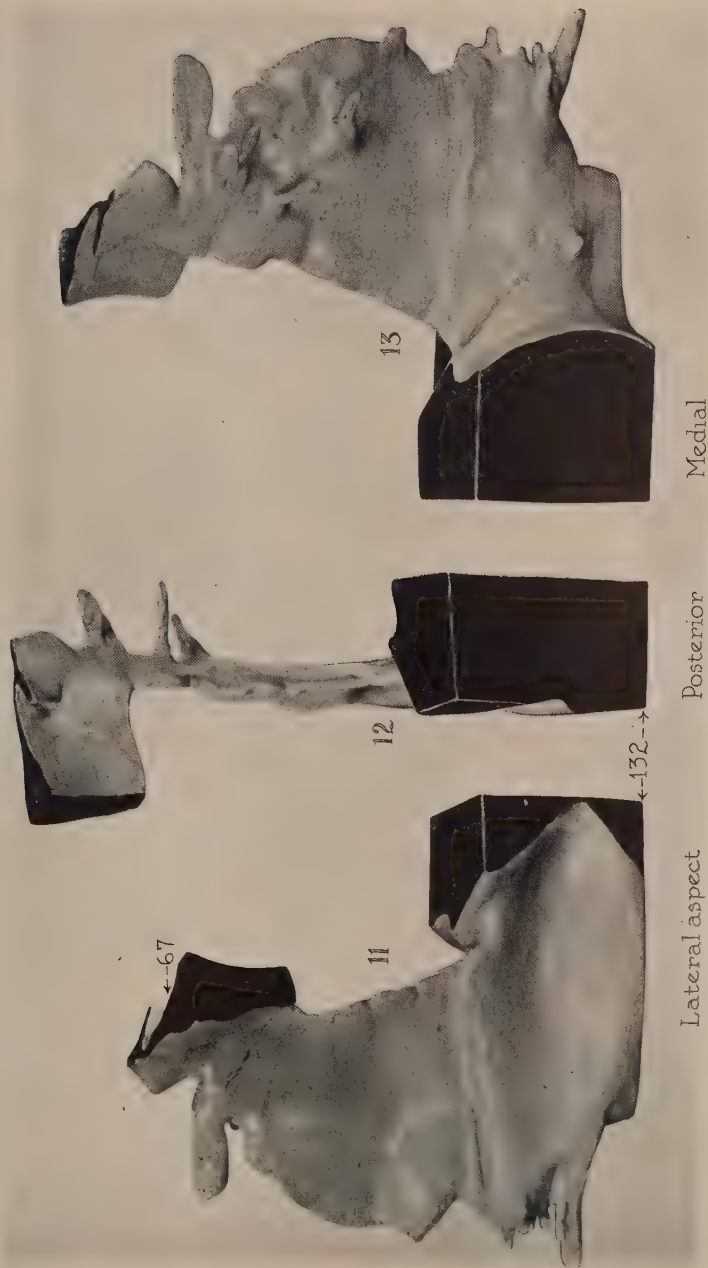
EXPLANATION OF FIGURES

Photographs (three views) of a model of the space enclosed by the walls of the fissula ante fenestram (sections 67 to 132, inclusive). The original model represents a magnification of 200 diameters; the photographs, of 60 diameters.

11 The model viewed from the lateral (tympanic) aspect; communications with the haversian systems are shown; the manner in which the long axis of the fissula changes from a coronal to a parasagittal plane is shown.

12 The model viewed from the posterior aspect (compare fig. 6), showing the manner in which the fissula communicates with the tympanic cavity (above and to observer's left) and with the vestibule (below and directed toward the observer). On the medial side the connections with haversian canals are shown.

13 The same model viewed from the medial (vestibular) aspect; in general, the outline is curved, both surfaces being concave backward. Communications between the vessels of the haversian canals and those of the fissula are numerous and best seen in this view.



THE EFFECT OF DICALCIUM PHOSPHATE, WITHOUT VITAMIN D, IN THE NUTRITION OF CHICKS

GEORGE M. HIGGINS¹

Division of Experimental Medicine

AND

CHARLES SHEARD²

*Division of Physics and Biophysical Research
The Mayo Clinic, Rochester, Minnesota*

SIX FIGURES

When chicks were grown in pens screened from sunlight by amber glass filters, which do not transmit the lesser wavelengths of sunlight, and were given food without vitamin D, skeletal abnormalities and hyperplasia of the parathyroid glands invariably developed. If 2 per cent by weight of cod liver oil was added to the diet, or if the chicks were housed behind vitaglass which transmits the ultraviolet wavelengths of sunlight, normal growth occurred. This overgrowth of the parathyroid glands has been interpreted as an attempt by the organism to compensate for the absence of vitamin D which facilitates absorption of calcium and makes it available for use. Leg weakness, low concentration of calcium in the blood, and other evidences of rickets developed in chicks not given food with vitamin D, although the calcium provided in the diet was adequate.

Recently, Bloom has reported that calcium in the form of refined dicalcium phosphate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) was utilized about 70 per cent more efficiently than tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$). The superiority of the absorption of dicalcium

¹ Associate Professor of Experimental Biology.

² Professor of Physics, The Mayo Foundation, University of Minnesota. Submitted for publication May 19, 1933..

phosphate was attributed to its greater solubility. The results showed that the utilization of calcium in adult rats was far greater when the dicalcium phosphate was substituted for the tricalcium phosphate. Bloom concluded that rickets is prevented and even cured, without vitamin D, by the substitution of dicalcium phosphate for tricalcium phosphate in the diet.

In view of this report and of our experience that tricalcium phosphate did not protect growing chicks from developing leg weakness in the absence of vitamin D, we have substituted pure dicalcium phosphate for the tricalcium and investigated such changes in the parathyroid and thyroid glands, the long bones and blood calcium and phosphorus as occurred in the first 3 months of life.

METHOD

In June, 1932, twenty-four White Leghorn chicks, 10 days old, were placed in a room screened from southern sunlight by an amber filter which transmits only the longer wavelengths. All chicks were provided a basic ration consisting of 55 per cent yellow corn, 17 per cent wheat middlings, 17 per cent meat scraps, 1 per cent salt, and 5 per cent sand grits. In addition to this basic ration, half of the flock (twelve chicks) received 4.9 per cent of the refined dicalcium phosphate, and the other half (twelve chicks) received 4.9 per cent of tricalcium phosphate. Thus the diets were alike, except in the form in which the calcium was provided. If dicalcium phosphate is more readily assimilable, then its utilization by the body should be shown in the growth and development of these chicks.

A third group of twelve chicks was placed in an adjoining room, behind a similar amber filter, and provided the same basic ration plus the 4.9 per cent of tricalcium phosphate, but, in addition, vitamin D was added to the diet in the form of 2 per cent by weight of cod liver oil. These chicks served as a control flock with which those in the other two pens were compared.

All chicks were weighed each week and two chicks from each flock were killed and examined at the end of the sixth, eighth, tenth, and twelfth weeks.

Determinations of the serum calcium and phosphorus were made at intervals throughout the experiment and the percentage ash determinations of the long bones were made at the end of the experiment. Histologic studies were made of the parathyroid and thyroid glands and of the femurs.

RESULTS

Growth and general appearance

The growth of the chicks in all three groups was essentially alike during the first 3 weeks (fig. 1). After the third week, the average growth of the control chicks exceeded that of the two groups given food with dicalcium and tricalcium phosphate, respectively, but without vitamin D. After the sixth week, chicks fed the dicalcium phosphate grew less rapidly than those fed the tricalcium phosphate. From the sixth week on, a wide discrepancy occurred in the average weight of chicks in these two groups. At the end of the investigation (12 weeks), the average increase in the weight of the control chicks was 29.5 ounces. The chicks fed the tricalcium phosphate had gained 19.1 ounces, whereas those fed dicalcium phosphate had gained only 14.0 ounces.

It is apparent from these growth curves (fig. 1) that chicks may be adequately maintained for a few weeks on diets with either dicalcium phosphate or tricalcium phosphate without cod liver oil. After the third week, however, chicks deprived of vitamin D failed to gain as rapidly and those receiving tricalcium phosphate (fig. 1, curve 2) increased in weight more rapidly than those fed the ration containing dicalcium phosphate.

The gross appearance of these chicks also indicated dietary deficiencies (fig. 2). In the control flock, provided with vitamin D, the chicks were all alert, their feathers were well preened, and heads and tails were carried erect. Marked contrasts to this appeared in both of the groups fed the di-

calcium and tricalcium phosphate without vitamin D. Chicks on the dicalcium diet were less attractive than those on the tricalcium diet. They were inactive and their feathers were ruffled and unkempt. Leg weakness and other signs of skeletal deficiencies developed.

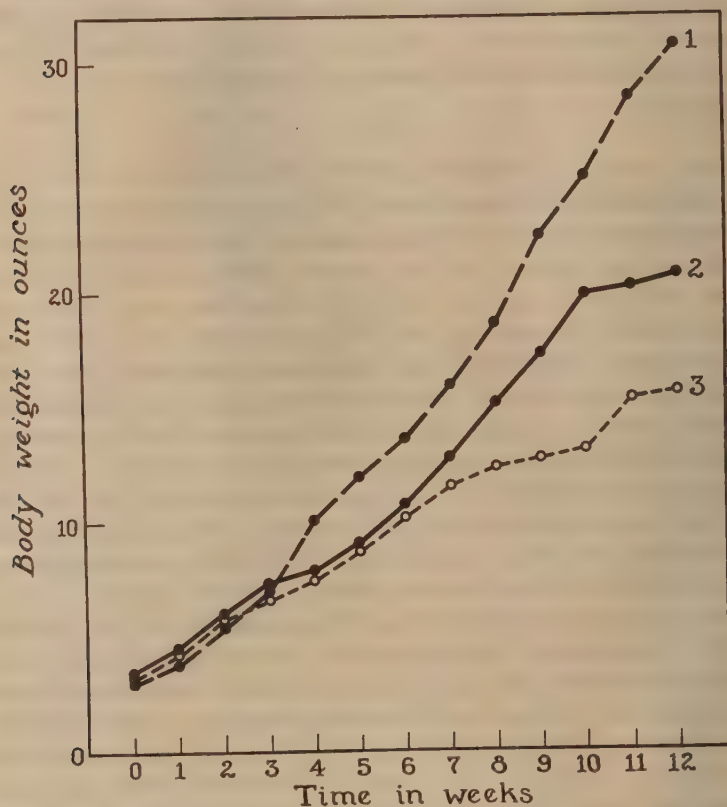


Fig.1 Growth curves of chicks. 1, control flock, vitamin D; 2, on tricalcium phosphate; 3, on dicalcium phosphate.

Serum calcium and phosphorus

The earliest determinations of serum calcium and phosphorus, made at the end of the sixth week, showed lower values for chicks fed the dicalcium than for those fed the

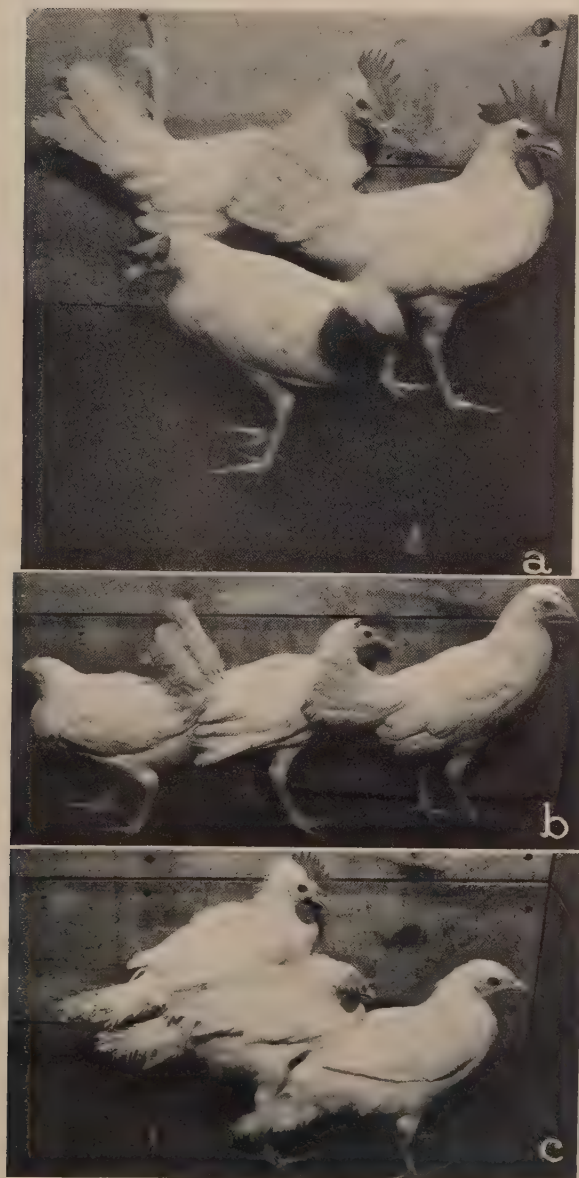


Fig. 2 Chicks, at end of 12 weeks; a, control chicks; b, chicks on tricalcium phosphate; c, chicks on dicalcium phosphate.

tricalcium phosphate, and these determinations were lower than those obtained for the control chicks, in which both serum phosphorus and calcium were well within the normal range. The average values of calcium and phosphorus determinations for three chicks taken from each of the three pens at the end of the investigation showed normal calcium and phosphorus in the blood of the control flock, lowered calcium in the blood of chicks fed the tricalcium, and the lowest calcium in those fed the dicalcium phosphate. The minimal value of calcium determinations occurred in chicks displaying the most marked skeletal lesions. It is probable that the excretion of calcium may have been greater in chicks on the dicalcium diet.

TABLE 1
Determinations of serum calcium and phosphorus at the end of 12 weeks

FLOCK	SERUM CALCIUM MG. PER 100 CC. BLOOD	SERUM PHOSPHORUS MG. PER 100 CC. BLOOD	RATIO OF SERUM CALCIUM TO SERUM PHOSPHORUS
Control	11.47 $\pm$ 0.27	5.27 $\pm$ 0.96	2.17
On tricalcium phosphate	8.55 $\pm$ 0.23	6.88 $\pm$ 1.16	1.24
On dicalcium phosphate	6.38 $\pm$ 0.21	6.51 $\pm$ 0.41	0.98

The values of the serum phosphorus were comparable in all groups, although higher in the two groups in which vitamin D was not given. With the higher phosphorus and the lower calcium values, the calcium-phosphorus ratios were those characteristic of rickets or leg weakness. The calcium-phosphorus ratio of 2.17 established for the blood of the control chicks was essentially normal; but the calcium-phosphorus ratios in the blood of chicks fed the tricalcium and dicalcium, 1.24 and 0.98, respectively, were those indicative of typical rickets (table 1).

The parathyroid glands

These glands in chicks are two small, discrete bodies at the posterior pole of each thyroid gland. Often they are at-

tached to the lateral surface of the thyroid gland or occasionally a diffuse parathyroid body confluent with the thyroid gland is encountered. Parathyroid cells are arranged in cords or columns and are separated from one another by strands of reticular fibers which form the supporting framework of the gland. From the larger intercolumnar fibers finer reticular fibers penetrate these cords of cells and form delicate networks about each parathyroid cell.

Six weeks after the beginning of this investigation, the parathyroid glands in control chicks were found to be normal. At the conclusion of the experiment (12 weeks), they were about 1.5 mm. in diameter and comparable cytologically with parathyroid glands of chicks grown on free range. Pathologic changes, however, developed in the parathyroid glands of chicks grown in both groups on the dicalcium and the tricalcium diets without cod liver oil. In our previous studies we invariably observed marked hyperplasia of all parathyroid bodies of rachitic chicks. On a dietary regimen in which vitamin D was withheld, but adequate calcium provided, lesions of bone were produced and the parathyroid glands were often six to eight times their normal size. Similar overgrowths occurred in these bodies in this investigation, but the pathologic changes were more extensive when chicks were maintained on the dicalcium diet (fig. 3). Even at 6 weeks, before there were gross manifestations of skeletal abnormality, the parathyroid bodies in the chicks on either the dicalcium or tricalcium ration were fully twice the size of those of control chicks. Degenerative changes had commenced, so that regression occurred simultaneously with proliferative hyperplasia. Degeneration was effected through the formation of small cysts and the infiltration of connective tissue, which, when revealed by silver stains, was greatly in excess of that in normal parathyroid glands.

When these cysts first appear in the hyperplastic gland they resemble keratin pearls and they recall the psammoma bodies of degenerating prostate glands or corpora amylacea bodies. This hyalin matrix, which stains a brilliant red with eosin,

is evidently a result of degenerative changes within the cytoplasm of the parathyroid cells. It is not colloid and does not appear to be a functional secretion of the gland. Small deposits were first identified within the lumens of small cords of cells and within the cytoplasm of the cells surrounding the lumen. With further regressive changes the amount of hyalin was increased and it assumed a concentric arrangement, so that in the larger pearls a definite whorl-like pattern was presented. Nuclei of the degenerated cells appeared as flattened plates between successive whorls of keratin. As



Fig. 3 Thyroid and parathyroid glands at end of 12 weeks; a, control chicks; b, chicks on tricalcium phosphate; c, chicks on dicalcium phosphate.

these cysts increase in size, they approach one another, often coalesce, and thus give rise to multiple cystic formations enclosed by a flattened squamous type of epithelium (fig. 4).

Thyroid glands

With the changes in the parathyroid bodies pathologic changes also develop in the thyroid glands. Although grossly the thyroid glands of chicks either on the secondary or the tertiary calcium diets appeared atrophic (fig. 3), yet actual hyperplasia had occurred. In the control group of chicks normal thyroid glands developed. The follicles were of the usual size, well filled with colloid, and the epithelium was flat

or low cuboidal. In chicks fed either the tricalcium or the dicalcium phosphate, marked changes occurred (fig. 5). The follicles were small, half to a third the size found in the normal gland, and the colloid was either absent or condensed into angles of the follicles. The epithelium was cuboidal or in many instances columnar and the nuclei were spherical rather than compressed as in normal follicles. There were many groups of undifferentiated thyroid cells in the interfollicular

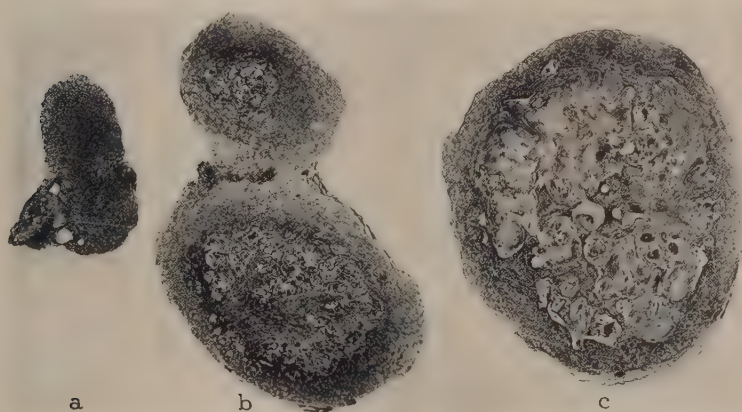


Fig. 4 Section of parathyroid glands at end of 12 weeks ($\times 60$); a, control chicks; b, chicks on tricalcium phosphate; c, chicks on dicalcium phosphate.

spaces, but the connective tissue stroma was no greater than that in normal glands.

The character of the follicle, the cuboidal or columnar epithelium, and the many groups of undifferentiated thyroid cells, all indicated hyperplasia. There is no doubt that proliferation of the thyroid cells occurred, but the elaboration of colloid by these cells was inhibited.

Skeletal changes

Skeletal abnormalities were not recognized until after the eighth week. Leg weakness and other signs of skeletal de-

iciency appeared in the two experimental flocks, but they were more marked in chicks feeding on dicalcium phosphate. The most marked deficiencies were the defective ossification

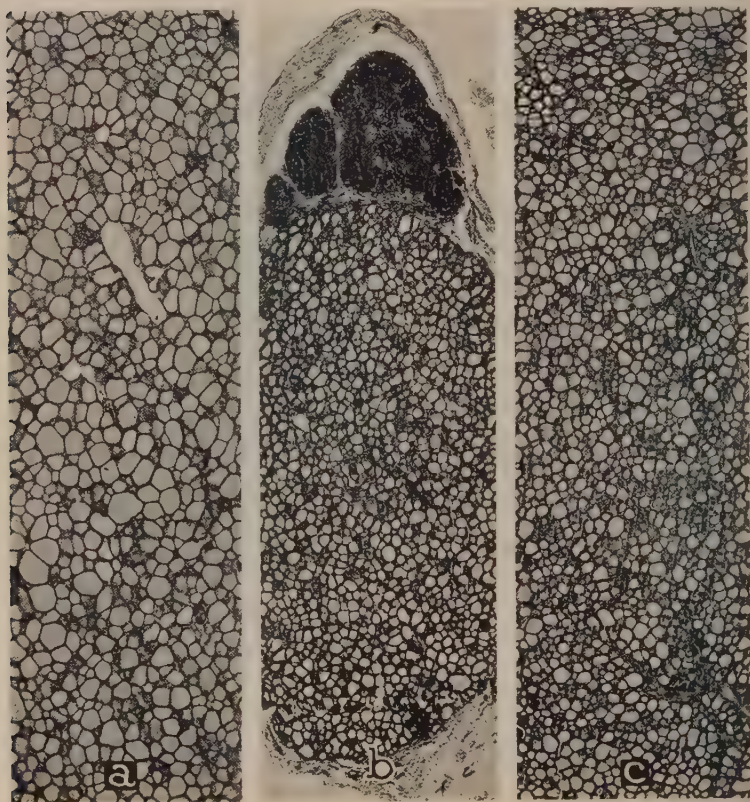


Fig. 5 Section of thyroid glands at end of 12 weeks; a, control chicks; b, chicks on tricalcium phosphate; c, chicks on dicalcium phosphate.

at the epiphyseal lines, the curved sternum, the enlarged articulations of the vertebral with the sternal ribs, and the enormous head and tubercle of each true rib articulating with the dorsal vertebrae. Sections of the long bones (fig. 6) showed clearly the absence of calcification which occurred in

chicks on the dicalcium phosphate diet without vitamin D. The appearance was not one of absorption, but of defective

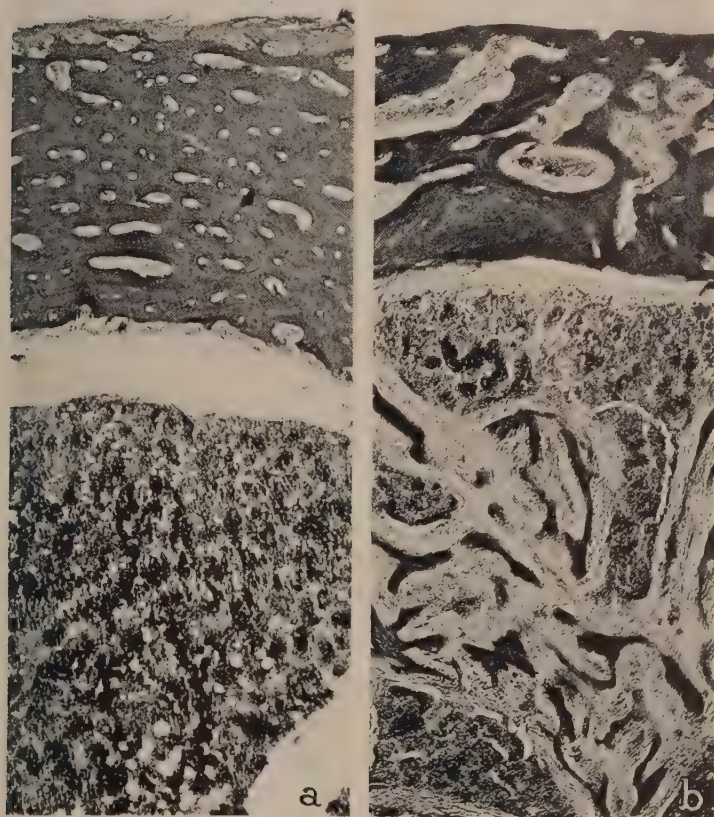


Fig. 6 Cross section of the femur of chicks at end of 12 weeks; a, control chicks; b, chicks on dicalcium phosphate.

calcification. There were extensive zones of osteoid tissue throughout the cortex, as well as in the marrow, and small areas of calcified tissue penetrated into the medullary portions. The appearance definitely indicated lack of calcium.

and thus verified the rachitic condition which was indicated by the low calcium phosphorus ratio of the blood. Low determinations of bone ash of the femurs of chicks at the end of the experiment showed the lack of mineral content in bones of those fed on the dicalcium salt. The lowest determinations were found in chicks maintained on the dicalcium ration, while those fed the vitamin D concentrate were highest of all and normal. So far as the deposition of calcium and phosphorus in bones of chicks is concerned, we are of the opinion that either dicalcium or tricalcium phosphate alone is inadequate and that vitamin D is essential to the proper utilization of either of these salts.

COMMENT AND SUMMARY

This investigation was undertaken to determine whether dicalcium phosphate would protect chicks from leg weakness and other skeletal defects without the presence of vitamin D. In our experience with growing chicks, rachitic conditions always developed when the diet contained adequate amounts of tricalcium phosphate, but no vitamin D. If the D factor was provided either in cod liver oil, viosterol, sunlight, or mercury arcs, all pathologic conditions were prevented. Thus the conclusion is warranted that the vitamin D concentrates were essential for the proper utilization of calcium.

As a sequel to the rachitic condition which develops in bones as a result of deficiency of vitamin D, hyperplasia of the parathyroid glands always occurs. Enormous overgrowths are often encountered, so that these bodies are as large or larger than the adjacent thyroid gland. These conditions have been described by Erdheim in rats and by Pappenheimer and Minor in children and they have been explained as an attempt, on the part of the organism, to compensate for a disturbed calcium metabolism. Klemperer believes that hyperfunction is not synonymous with hyperplasia, but rather regards these overgrowths as the structure of a hypofunctioning gland. On the other hand, parathormone, the active principle of the parathyroid gland, does not protect chicks against rachitic

symptoms, although there were indications that it did inhibit so marked an overgrowth of the gland. Thus one may infer that the hyperplastic gland does actually elaborate an excess of the active principle; but in the absence of vitamin D, normal calcium metabolism is interrupted.

With this hyperactivity of the gland and increased elaboration of the principle a hyperparathyroid condition is induced. Recently, Oberling and Guérin described the lesions of osteitis fibrosa in chicks with greatly enlarged parathyroid glands, and showed that the lesions of rickets and the lesions of osteitis fibrosa may develop at the same time and are not readily distinguished from each other. Clinically and experimentally, osteitis fibrosa occurs as the result of extensive decalcification of bone induced by parathormone in excess. Rickets is produced by defective deposition of calcium or disturbed ossification. It is quite clear that these two conditions, although not comparable, may go on synchronously in the same region and produce extensive changes in bone. Our observations agree with those of Oberling and Guérin.

On the other hand, our observations of chicks do not conform to the observations of Bloom on rats. Not only in utilization, but in calcification and in solubility, he found that the dicalcium phosphate is far more effective than the tricalcium phosphate. Rachitic conditions were not only prevented, but were cured, when dicalcium phosphate was fed rats without any of the vitamin D concentrates. The growth curve, the gross appearance of the chicks throughout the experiment, the ratio of serum calcium to serum phosphorus, the pathologic conditions induced in the parathyroid and thyroid glands and the long bones, all indicated that better growth and development took place in chicks fed tricalcium phosphate than in those fed the dicalcium phosphate. We have no explanation for the discrepancy in these results. It may be correlated, however, with differences in the physiology of the gastro-intestinal tracts of birds and mammals. In view of the clinical significance involved, further experimental work must be done to appraise more completely the degree of the utilization of these two calcium salts.

CONCLUSION

Dicalcium phosphate, without vitamin D, is not adequate to maintain growing chicks and will not protect against leg weakness and other skeletal deficiencies which arise as a result of a disturbed calcium metabolism.

LITERATURE CITED

- BLOOM, C. J. 1932 Secondary calcium phosphate prevents and cures rickets without vitamin D. I. Utilization studies. II. Calcification studies. III. Solubility studies. *Proc. Soc. Exper. Biol. and Med.* vol. 29, pp. 860-861; 861-863; 864-865 (April).
- ERDHEIM Quoted by Pappenheimer and Minor.
- HIGGINS, G. M., W. I. FOSTER, AND CHARLES SHEARD 1930 Further investigations on the effects of radiant energy on the development of the parathyroid glands of chicks. *Am. J. Physiol.*, vol. 94, pp. 91-100 (July).
- HIGGINS, G. M., AND CHARLES SHEARD 1928 The effects of selective solar irradiation on the parathyroid glands of chicks. *Am. J. Physiol.*, vol. 85, pp. 299-310 (June).
- HIGGINS, G. M., R. M. WILDER, AND CHARLES SHEARD Unpublished data.
- KLEMPERER, PAUL 1923 Parathyroid hyperplasia and bone destruction in generalized carcinomatosis. *Surg. Gynec. and Obst.*, vol. 36, pp. 11-15 (Jan.).
- OBERLING, C., AND M. GUÉRIN 1931 Lésions d'ostéite fibreuse chez la poule, avec hypertrophie des parathyroïdes. *Compt. rend. Soc. de biol.*, T. 108, pp. 1134-1136 (Jan. 8).
- PAPPENHEIMER, A. M., AND JOHN MINOR 1921 Hyperplasia of the parathyroids in human rickets. *J. Med. Res.*, vol. 42, pp. 391-403 (June-Sept.).
- SHEARD, CHARLES, AND G. M. HIGGINS 1928 The effects of selective solar irradiation on the growth and development of chicks. *Am. J. Physiol.*, vol. 85, pp. 290-298 (June).
- SHEARD, CHARLES, G. M. HIGGINS, AND W. I. FOSTER 1930 The growth and development of chicks as influenced by solar irradiation of long visible and ultraviolet wavelengths, respectively, with and without supplementary irradiation of various types. *Am. J. Physiol.*, vol. 94, pp. 84-90 (July).

NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

PSYCHOLOGY APPLIED, by George W. Crane. 1933. Size, $5\frac{1}{2} \times 8\frac{1}{2}$ inches. 586 pages. Index. Lists of references. Illustrated. Cloth. Price, \$4.00. Northwestern University Press, Chicago.

There is nothing vague and rambling about this book. Doctor Crane uses the psychology he teaches. He stresses the principle that specificity produces a more vivid response than generality, and throughout the book backs up theories with illustrative examples and cases. He does not merely say that a person should develop social intelligence, but he gives definite advice on how to do it. He does not state vaguely that successful advertisements are those which capture and hold the attention, but he analyzes advertisements and points out the factors that make them effective. From his familiarity with the problems of students and of older men and women he has found it desirable to include chapters on phases of applied psychology not usually treated in standard textbooks. They are the psychology of improving your personality, the psychology of music and morale, the psychology of the public platform, the psychology of writing and art, and child psychology. Any reader concerned with his relation to the world about him will be interested, entertained, and enlightened by reading this book.

CRIMES AND CRIMINALS, by William A. White. 1933. Size, $5\frac{1}{2} \times 8\frac{1}{2}$ inches. Pages viii + 276. Index. Cloth. Price, \$2.50. Farrar and Rinehart, Inc., New York.

This is an essay on human relations illustrated with material and case histories taken from the field of criminology. It presents man as a social animal and describes the structure and function of mind, with definitions of new concepts recently introduced in psychology—ego, id, super-ego, determinism, emotional genesis, the unconscious, conflict, psychogenesis, and distorting mechanisms. It studies the antisocial mind, normal and abnormal, investigates heredity and environmental factors causing it, and sets forth the author's points of view on prevention, treatment, and punishment of crime. Doctor White's long experience and prominence as a psychiatrist guarantee the soundness and importance of the book, and the clearness and vividness of his writing make it understandable and interesting to any intelligent reader.

COLONY FOUNDING AMONG ANTS, with an Account of Some Primitive Australian Species, by William Morton Wheeler. 1933. Size, $5\frac{1}{2} \times 8$ inches. Pages x + 179. Index. Bibliography. 29 illustrations. Cloth. Price, \$2.00. Harvard University Press, Cambridge.

A new method of colony founding behavior in the Ponerine ants in Australia was observed by the author. This surprisingly unexpected method proves to be a significant link connecting the behavior of ants with that of solitary and social wasps, and shows that the queen ant has far more initiative than she has been credited with in the past. Her behavior is a valuable confirmation of the importance of the mother-family concept in the study of social insects. This book gives a technical review of all known methods of colony propagation, taxonomic data, and natural history notes on bulldog ants and other primitive Australian genera.

NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

PHYSICAL CHEMISTRY OF LIVING TISSUES AND LIFE PROCESSES, As Studied by Artificial Imitation of Their Single Phases, by R. Beutner. 1933. Size, $6\frac{1}{4} \times 9\frac{1}{4}$ inches. Pages x + 337. 79 illustrations. Subject and author indices. Cloth. Price, \$5.00. The Williams and Wilkins Company, Baltimore.

Many attempts have been made to bridge the gap between inanimate and living matter, and many of the single phases of vital activity have been artificially imitated. Accounts of such investigations scattered throughout scientific literature have been collected by the author and published together with his own original experiments never before reported. Three attempts at approach are described and in each vital functions are analyzed by means of the following steps: 1) The finding of suitable artificial models which reproduce certain phenomena peculiar to living organisms. 2) The study of the physical laws of the model. 3) The application of these physical laws to biological problems. 4) Finally, on the basis of all the experience gained, an extension of the experiments with models attempted. The first attempt at approach is entitled 'Membranes, osmosis and related forces.' The second attempt, 'Life processes related to crystallization or due to surface forces'; the third, 'Electrical currents in tissues and their relation to life processes.' The last chapters view the future possibilities of development. New investigations on membrane equilibria are printed in the appendix.

BIOLOGY, Extracted from Vol. VIII (1927-1928) and IX (1929) of the Annual Tables of Constants, by Prof. E. Terroine (Strasbourg) et Dr. Janot (Paris). 141 pages. 22cm. $\times$ 28 cm. Price, bound, francs, 90 (\$3.52). Publisher, Gauthier-Villars & Co., Quai des Grands Augustins, Paris.

Tabular matter of interest in the field of biology has been selected from more than 600 cooperating publications. Volume 8, covering 1927 and 1928, and Volume 9, covering 1929, are bound together as one book. The subject matter of each volume is divided as follows: 1) General properties of living beings. 2) Animal Biology. 3) Vegetable Biology.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN, Lieferung 409. Edited by Geh. Med.-Rat. Prof. Dr. Emil Abderhalden. Abt. VIII. Methoden der Experimentellen morphologischen Forschung, Teil 1, Heft 10. 1933. Size, 7×10 inches. Price, RM 4.20. Urban und Schwarzenberg, Berlin and Vienna.

H. J. Arndt. Untersuchungsmethoden der Epithelkörperchen. 3 illustrations. H. J. Arndt and H. O. Neumann. Untersuchungsmethoden der Bauchspeicheldrüse. 3 illustrations. Herbert Siegmund. Die morphologischen Methoden zur Untersuchung der Nebenniere und des chromaffinen Systems (Paraganglien).

NEW BOOKS AND PUBLICATIONS

THE MECHANISM OF NERVOUS ACTION. Electrical Studies of the Neurone, by E. D. Adrian. 1932. Size, $6 \times 9\frac{1}{2}$ inches. Pages x + 103. 35 illustrations. Index. Bibliography. Cloth. Price, \$2.00. University of Pennsylvania Press, Philadelphia.

These studies deal with the use of the thermionic valve for amplifying nerve action currents, and illustrate the chief lines of work carried out by this technique. They are mainly concerned with the behavior of the units which make up the nervous system. The first chapter sketches the development of electrophysiology from the first experiments of Galvani to the membrane hypothesis. The second chapter takes up the action of the sense organs—adaptation, production of rhythmic discharge, depolarization, injury discharge, etc. The third chapter discusses pain, tactile endings, protopathic and epicritic fibers, and specific nerve impulses; the fourth, the discharges in motor fibers; and the last chapter, activity of nerve cells, automatic rhythm, potential changes and gradients, and synchronous activity.

NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

LYMPHATICS, LYMPH, AND TISSUE FLUID, by Cecil K. Drinker and Madeleine E. Field. 1933. Size, $5\frac{1}{2} \times 8\frac{1}{2}$ inches. Pages xv + 254. 14 illustrations. Bibliography. Subject and author indices. Cloth. Price, \$3.00. The Williams and Wilkins Company, Baltimore.

The authors have summarized in the present volume such facts as are known about the lymphatic system, a task for which they are well fitted as a result of their own direct experimentation in the field. The data presented are drawn from anatomy, physiology, immunology, and pathology; and taken as a whole they give a working idea of what the lymphatics are steadily engaged in doing in the mammalian body. The chapter headings are as follows: The structural basis of the lymphatic system. The entrance of foreign particles and colloidal solutions into lymphatics. The permeability of the blood capillaries. The limits of the permeability of the typical normal blood capillary to colloids. The flow of lymph. The composition of lymph. The tissue fluid. Practical considerations.

THE DOG'S MEDICAL DICTIONARY, An Encyclopedia of the Diseases, Their Diagnosis and Treatment, Poisons and Their Antidotes, and the Physical Development of the Dog, by Alfred J. Sewell, revised and largely rewritten by Frederick W. Cousens. 1933. Size, $5\frac{1}{2} \times 8\frac{1}{2}$ inches. Pages xii + 324. Illustrated. Cloth. Price, \$2.75. Charles Scribner's Sons, New York.

To veterinarians, owners of dogs, and to researchers using the dog as an experimental animal, this is a most useful book. It describes all the ailments a dog is likely to be afflicted with, and gives advice on feeding and general care. For convenient reference, subjects are listed alphabetically, from abdomen to wounds, and adequate consideration is given to each subject, whether it be disinfecting of kennels, bee stings, fractures, or major operations. Descriptions of symptoms and treatment of diseases enable a curator to recognize sickness in his animals and to give the right treatment. Prompt identification and treatment of disorders may save the lives of valuable dogs irreplaceable in a series of experiments. The book is beautifully illustrated with photographs of prize-winning dogs of many breeds.

VERTEBRATE PALEONTOLOGY, by Alfred Sherwood Romer. 1933. Size, 7×10 inches. Pages vii + 491. 359 illustrations. Index. Bibliography. Synoptic Classification of Vertebrates. Cloth. Price, \$5.00. The University of Chicago Press, Chicago.

In appreciation of the fact that this book may be read by all kinds of readers, the author has supplied in his first chapter certain fundamentals of biology and geology necessary for understanding the material which follows. It includes a short discussion of fossils and how they have been preserved, the geological time scale, taxonomy and classification, a condensed description of vertebrate

NEW BOOKS AND PUBLICATIONS

structure, vertebrate ancestry, and classification of vertebrates. After this introduction it begins with the primitive jawless vertebrates, such as the lampreys, and traces the increasing complexity of structure through fishes, amphibia, reptiles, mammal-like reptiles, and mammals from the simplest to the primates. There are many pictures and descriptions of aberrant lines of development and curious end forms, as well as the main line along which modern animals have developed. The book ends, of course, with a chapter on primates, with particular emphasis on man, and a description of fossil human types: Java ape man, Piltown man, Peking man, Heidelberg man, Neanderthal man, and modern man.

THE PHYSIOLOGICAL EFFECTS OF RADIANT ENERGY, by Henry Laurens. 1933. Size, $6\frac{1}{4} \times 9\frac{1}{4}$ inches. 610 pages. 104 illustrations. Bibliography. Subject and author indices. Cloth. Price, \$6.00. The Chemical Catalogue Company, Inc., New York.

This monograph is one of the series published by the American Chemical Society. The purpose of this series is twofold: to present in readable form all available knowledge on a chosen subject, making it intelligible to those whose activities are along a different line, and to stimulate research along similar lines by surveying the progress already made and pointing out directions in which the investigation should be extended. The choice of subject for this monograph is most appropriate because of the clinical importance of sunlight and ultraviolet rays in the treatment of tuberculosis, tetany, rickets, and other diseases. There is a tendency now to exaggerate the vital importance of sunlight and to regard it as a cureall, whereas there is little if any reason for supposing that the growth of the higher animals is dependent upon light, although it may produce physiological effects when appropriate conditions for its influence present themselves. This timely book views all aspects of the subject—its effect on the structures and functions of the body, and its mode of action on physiological and pathological processes.

ATLAS ANATOMICUM CEREBRI HUMANI, by G. Jelgersma. Size, $13\frac{1}{2} \times 18$ inches. Scheltema and Holkema's Boekhandel, Amsterdam.

The atlas consists of a series of 108 separate plates picturing 168 sections of the human brain. The plates are reproduced by heliotype process from photographs of stained preparations. They are designed particularly for study and demonstration of the course of fibers and fiber tracts. A continuous set of serial sections of the human brain in the three dimensions of space is difficult to obtain and many laboratories will find this atlas, made with careful attention to detail, an acceptable substitute. For some purposes it may be preferred, since the photographs are more easily handled than the sections themselves. Each plate is provided with a transparent cover page on which is traced the outline of the section and a network of vertical and horizontal lines. These lines are lettered and numbered and form guides for locating structures. The plates are indexed as follows: Series Frontalis Hemispherii, 1-45; Series Trunca Cerebri, 46-67; Series Sagittalis Systemae Nervosae Centralis, 68-74; Series Horizontalis Hemispherii, 75-81; Series Meynert, 82-105; Medulla Spinalis, 106-108.

NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

TEXTBOOK OF GENERAL ZOÖLOGY, Second Edition, by Winterton C. Curtis and Mary J. Guthrie, with the Collaboration of Katherine R. Jeffers. 1933. Size, $6 \times 9\frac{1}{4}$ inches. Pages xv + 588. Index. Glossary. 438 illustrations. Price, \$3.75. John Wiley and Sons, Inc., New York.

In any brief textbook of general zoölogy the authors are faced with the problem of selecting from a wealth of material those facts and methods which will give the student a satisfactory working knowledge of the subject within the time limits allowed for the course. In the present book the authors have chosen wisely to approach the subject through a study of vertebrate anatomy, since the facts closely related to human structure and function are more familiar and more interesting to the student. After this beginning, principles of animal life are presented in the formal manner current in most textbooks. The final chapters discuss development, genetics, and evolution. The text is written in a clear and pleasing style readily comprehended by educated adults. The binding of the volume is waterproof and vermin proof, a desirable feature in books frequenting laboratories.

KREISLAUFSTÖRUNGEN UND PATHOLOGISCHE HISTOLOGIE, by Martin Nordmann. (Ergebnisse der Kreislaufforschung, Band IV.) 1933. Size, $6\frac{1}{4} \times 9\frac{1}{4}$ inches. Pages x + 174. 14 illustrations. Bibliography. Subject and author indices. Price, RM 13.80; bound, RM 15.00. Theodor Steinkopff, Dresden and Leipzig.

This volume is divided into two parts. Part one, which describes diseases of the circulatory system, is based on observation and clinical study of patients. Part two, dealing with pathological histology, is based on dissected material and cadavers. Results of experimental work carried out on animals have been used to supplement human material.

TRANSACTIONS OF THE BOSE RESEARCH INSTITUTE, CALCUTTA. A Record of Research carried on in Various Branches of Science. Volume VII, 1931-1932. Edited by Jagadis Chunder Bose. 1933. Size, 6×9 inches. Pages vi + 343. 161 illustrations. Buckram. Price, \$8.50. Longmans, Green & Co., New York, London, and Toronto.

This volume continues a series formerly issued under the title of "Life Movements in Plants." The title has been changed because this series of papers contains reports of research in other branches of science besides plant physiology. They include a study of the effects of certain salts and native plant extracts on fish and frogs, a comparative study of Burmese crania, a description of the behavior of fish-eating spiders, and an investigation of radio-activity of Hot Springs at Rajgir.

